Northern Provenances of Silver Fir Differ with Acclimation to Contrasting Light Regimes

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Abstract: Phenotypic differentiation of Abies alba was identified among selected provenances originating from the northern edge of this species’ natural geographic range. We posited that although the seed sources of the study populations were not geographically distant, progenies would differ with respect to growth, needle pigment concentration, and values of photochemical parameters in response to contrasting light conditions. Potted seedlings of six provenances were grown for two seasons under low light (LL, 40% of full irradiance) or high light (HL, 100% of full irradiance). The results showed that the provenances differed in diameter at root collar (DRC) and in relative growth rates (RGR). Seedlings grown in full light had a greater mean value of DRC than those in LL. LL increased total needle chlorophyll (Chl_{tot}) and carotenoid concentrations compared with HL, and Chl_{tot} was modified by the provenance of the seedlings. The provenance “Syców”, localized at the northern limit of the species range, showed the lowest value of maximal electron transfer rate (ETR_{max}) and lower values of growth parameters compared with the other study provenances. Relative growth rate, Chl_{tot}, and non-photochemical quenching of fluorescence (NPQ) were correlated with some climatic characteristics of seed stands and formed clusters indicating the local adaptation of the study populations. These results provide evidence of low intraspecific A. alba differentiation in terms of growth dynamics, photochemical capacity, and tolerance to different light regimes. The intraspecific diversity found in our study among the northern lead populations could be, however, important for the natural expansion of this species further north and for the selection of provenances to be used in the programme of the A. alba restoration in the Sudety Mountains, southern Poland, where decline of this species has been observed.

Keywords: Abies alba; chlorophyll a fluorescence; ecotype; leaf photochemistry; photosynthetic pigments; species restoration

1. Introduction

Intraspecific diversity of species is as important for biodiversity as interspecific diversity; however, this area has been overlooked [1]. The genetic diversity of trees can be revealed by investigating phenotypic variability, i.e., by determining the differences between populations originating from different regions [2].

High intraspecific diversity and high number of ecotypes resulting from genetic diversity can facilitate the adaptation of a species to global climate changes. Moreover, the use of selected reproductive material for forest regeneration will be essential for mitigating the negative and enhancing the positive impacts of climate change on managed forests [3,4]. It is crucial to know the suitability of the tree species’ provenance to the expected conditions, which depends to a large extent on its physiological makeup.

Our study species, Abies alba Mill., distinguishes itself from other European species by its high shade tolerance. At the juvenile stage, it can grow at extremely low light levels of
5%, and at the age of 5 years it requires 18% of full irradiance [5]. Seedlings of A. alba show remarkable growth and photosynthetic plasticity in response to the light environments under canopies of different forest tree species [6]. The suitable light conditions are crucial for growth of the natural and artificial regeneration of A. alba; however, its intraspecific differentiation in response to light has not been fully elucidated. Our study species is regarded as being more tolerant to drought than Picea abies, but less tolerant than Pinus sylvestris L. or Quercus petraea [7,8]. A mean annual rainfall of 700 mm limits the natural range of A. alba in the north. The optimal annual rainfall exceeds 1500 mm and the mean annual temperature is about 9 °C [9]. Abies alba does not tolerate minimum temperatures below −20 to −25 °C. Low temperatures in winter, spring frosts, and water deficits are the main factors that determine the northern and eastern limits of its natural range. In Poland, this species grows naturally in different types of soil ranging from podzolic, brown, and lessivé, to peat soils in mountains and uplands, from 500 to 1100 m a.s.l., although some isolated populations occur in lowlands [9,10].

Abies alba produces heavy pollen and seeds, thus it cannot spread over long distances, which suggests that geographically isolated populations can be characterized by low gene flow, high inbreeding, and low genetic diversity; however, genetic diversity does not necessarily decrease from the core to the periphery (reviewed by [11]). In the Carpathian Mountains, inter-population diversity of A. alba is high; however, the Sudety Mountains populations (southern Poland) show much lower genetic diversity [12]. At the northern edge of its natural range, A. alba grows in small and often topographically separated populations that are adapted to local site conditions. In these populations, evolutionary processes such as adaptation, genetic drift, gene flow and selection have occurred [13,14].

There exists limited information about the genetic and phenotypic diversity and ecological plasticity of A. alba populations from the northern edge of its natural range, where the decline of this species has been observed [14]. The results of one of the oldest provenance trials with A. alba showed that seed sources from southern Italy (Calabria) were characterized by higher variability and vitality compared with provenances from central and northeastern Europe [15]. Analyses of isoenzymes indicated low physiological and evolutionary adaptive potential in the central and northeastern populations [16]. Functional intraspecific variation among northern montane provenances and within study populations in ability to acclimate to light and temperature was evidenced; however, altitudinal ecotypes associated with climatic variables were not identified [17]. In provenance trials with A. alba localized in Austria and Slovakia, evidence was provided that PSII thermostability was higher in Balkan provenances compared with in northern ones [18]. Short nucleotide polymorphism analyses conducted on the same trees showed significant associations with latitude or bioclimatic variables and needle osmotic potential [19].

In Central Europe, A. alba has been threatened by decline caused by air pollution, mainly SO2 and NO3 (acid rains), which damages the photosynthetic apparatus [20], toxic Al3+ ions affecting the roots [21], warmer climate with drought episodes that are more frequent and longer than usual, browsing by ungulates, low genetic variation among provenances, and other reasons that have not been fully elucidated [22,23]. Sylvicultural systems applied in Europe are thought to be among the main reasons for its disappearance or significant decline in some areas [23]. Since the 1980s, recovery following A. alba regression has been observed, which was revealed by a higher annual ring increment and abundant natural regeneration [23,24]. Recently published bioclimatic models predict that A. alba will expand its natural geographic range far to the north, and in the face of global warming, it may outcompete P. abies, which is less tolerant to drought [25]. Knowledge about the ecotype variation and plasticity of A. alba, which was threatened by decline in Central Europe, is paramount for its conservation and restoration.

In the present study, we focused on A. alba seedlings originating from seed stands growing at the northern limit of the species’ geographical range or outside this limit in order to determine the differentiation of growth parameters and leaf photochemistry among the study populations under contrasting light regimes. Some of these populations have
already been used as valuable seed sources for the restoration of *A. alba* in the Sudety Mountains [26].

The values of the photosynthetic parameters of the Carpathian and the Sudety Mountains *A. alba* populations from southern Poland are weakly correlated with the altitude of origin [17]. We expanded on this by using not only mountainous, but also lowland provenances, including one from outside of the natural range of *A. alba*, to investigate the ability of seedlings to acclimate their photosynthetic machinery to irradiance. When geographically closely situated plant populations are compared, as in our present study, it can be expected that phenotypic and genetic differentiation will be subtle; however, a strong correlation between significant environmental heterogeneity and genetic heterogeneity in small spatial scales has been documented for seed characteristics, leaf traits, phenology, physiological traits, breeding systems, etc. (reviewed in [27]).

In this study, we addressed whether silver fir populations from the northern edge of the species’ geographical range were adapted to their local site conditions and formed local ecotypes that differed from each other with respect to growth and photochemistry. The outcomes of an ongoing systematic review suggest that the question about differentiation in needle photochemistry and photosynthetic pigment concentration among northern and outside-the-range populations of *A. alba* has not been empirically addressed to date, except for the results from provenance trials [18]. We hypothesized the following: (1) Silver fir would acclimate to the experimental light conditions in terms of relative growth rate (*RGR*), photochemistry, and in needle pigment concentration. (2) The provenances of seedlings used in our experiment would differ in growth and photochemical parameters due to the local adaptation of the mother trees, despite the relatively small geographic distances among the seed stands. In particular, in our pot experiment, the montane provenances were expected to show lower growth rates and a higher needle chlorophyll concentration than lowland provenances, as was shown for *P. abies* seedlings originating from lower and upper montane zones [28]. Differences among the study provenances would be modified by their specific ability to photochemically acclimate to the study light environment. (3) Alternatively, the study provenances would not remarkably differ in terms of phenotypic traits, suggesting low inter-provenance diversity and low potential adaptability to changes in climate.

2. Materials and Methods

2.1. Materials

In October 2016, in Poland, cones of *Abies alba* (Mill.) were collected in six forest districts: M—“Miedzylesie”, KG—“Kamienna Góra”, S—“Syców”, K—“Kolumna”, B—“Brzeziny”, and O—“Osusznica”. The names of the *A. alba* provenances were created from the names of the Forest Districts of the seeds’ origin (Table 1). The age of mother trees was in the range from 115 to 132 years. In the nursery of the “Jarocin” forest district (5°58' 45.46" N, 17°29'9.996" E), seeds of each study provenance were stratified at 8 °C in dark over 2–5 months depending on the provenance; “Osusznica” seeds germinated after only two month, and “Syców” seeds after 5 months under stratification. S showed the lowest (55%), and O the highest (90%), germination rates. Germinating seeds were sown into containers with cells of 350 cm³. There were five containers per provenance and 52 cells in each container. The containers were placed in an unheated plastic tunnel where seedlings were grown to August 2017 and then placed out of the tunnel, on the ground, 40 m from the forest edge. Seedlings were fertilized with 3 g of Osmocote per cell mixed with substrate (peat:perlite; 3:1; v/v) and watered in spring and summer to maintain the volumetric substrate moisture at 22%–30%. In April 2019, containers with seedlings were transported to the Dendrological Garden of the Poznań University of Life Sciences. Seedlings were taken out from containers and immediately planted in 10 L pots filled with a 2 cm layer of gravelite and a mixture of sand and peat (4:1; v/v).
Table 1. Climate characteristics of seed stands where Abies alba cones were collected.

| Provenance | Latitude, Longitude | Altitude a.s.l. (m) | $T_{\text{mean}}$ (°C) | $T_{\text{max}}$ (°C) | $T_{\text{min}}$ (°C) | $P_s$ (mm) | $P$ (mm) | $\text{AHM}$ | $\text{SH}$ |
|------------|---------------------|---------------------|------------------------|------------------------|------------------------|-----------|-----------|-------------|-------------|
| Międzybiesie | 50°17'34.3" N 16°43'37.1" E | 450 | 7.8 | 17.1 | -2.2 | 397 | 636 | 28.0 | 37.1 |
| Kamienna Góra | 50°36'51.5" N 15°59'22.3" E | 640 | 6.5 | 16.1 | -4.4 | 417 | 707 | 23.3 | 55.2 |
| Syców | 51°21'31.3" N 17°36'32.9" E | 201 | 8.9 | 18.7 | -1.6 | 321 | 551 | 34.3 | 65.1 |
| Kolumna | 51°34'14.8" N 19°20'49.6" E | 220 | 8.9 | 19.9 | -3.3 | 320 | 554 | 34.1 | 63.8 |
| Brzeziny | 51°40'57.0" N 19°44'53.0" E | 217 | 8.6 | 19.7 | -3.8 | 319 | 552 | 33.7 | 63.9 |
| Osuszniczka | 54°6'27.24" N 17°23'25.8" E | 221 | 7.7 | 18.4 | -3.9 | 364 | 708 | 25.0 | 51.9 |

The study provenances are listed from the most southern to the most northern provenance. $T_{\text{mean}}$—mean annual temperature; $T_{\text{max}}$—mean maximal temperature of the warmest month; $T_{\text{min}}$—mean minimal temperature of the coldest month; $P_s$—mean summer sum of precipitation; $P$—mean annual sum of precipitation; $\text{AHM}$—mean annual heat:moisture index $[(T_{\text{mean}} + 10)(P/1000)]$; $\text{SH}$—mean summer heat:moisture index $[T_{\text{max}}/(P/1000)]$. Meteorological data are the mean values from the period of 30 years (1991–2020), and come from ClimateEU software package [29].

2.2. Site Conditions of Provenance Selection

Seedlings of six $A.\ alba$ provenances from southern (M, KG), central (S, K, B), and from the outside of the species’ natural range in northern Poland (O) were used in our experiment (Table 1). The seed stands of seedlings’ origin differ with respect to their climate and soil conditions. The seed stand KG is situated at the highest altitude of 640 m a.s.l., and S at the lowest altitude of 201 m a.s.l., among the study provenances. The difference between the highest and lowest values of mean annual temperature is 2.4 °C (S, K, and KG). Differences between the values of extreme temperatures—mean maximal temperature of the warmest month ($T_{\text{max}}$) and mean minimal temperature of the coldest month ($T_{\text{min}}$) are 3.8 and 2.8 °C, respectively. The montane provenances (M and KG) have the highest values of mean summer sum of precipitation ($P_s$) and mean annual sum of precipitation ($P$) among the study provenances, and S, K, and B have the lowest sums of precipitation. Interestingly, O, which is the most northern provenance, and closest to the Baltic seaside, has almost the same mean annual sum of precipitation as the montane KG. The mean values of the annual heat:moisture index ($\text{AHM}$) indicate that the stands situated in central Poland (S, K, B) are more threatened by drought than the montane provenances (M, KG) and the O population (Table 1). In central Poland, however, the air temperature and precipitation, which are less suitable for $A.\ alba$, can probably be mitigated by the high water retention of soil rich in clay (Table 2). M and O are the most widely distant populations. The distance between them is around 549 km. The latter is outside the natural geographic range of $A.\ alba$. Its origin was identified using mitochondrial and chloroplast DNA markers as being the Sudety Mountains (SW Poland) [30].

Table 2. Soil conditions of the stands where Abies alba cones were collected.

| Provenance | Forest Site Type | Soil Type | Soil Valuation Item |
|------------|-----------------|-----------|---------------------|
| Międzybiesie | Fresh mixed mountain forest | Acid brown soil | Loamy silt |
| Kamienna Góra | Fresh mixed mountain forest | Brown podzolic soil | Loamy gravelly sand |
| Syców | Upland fresh forest | Acid brown soil | Clay loam |
| Kolumna | Fresh forest | Pseudogley soil lessive | Loamy sand, sandy loam |
| Brzeziny | Fresh forest | Soil lessive | Boulder clay, post-glacial sand |
| Osuszniczka | Fresh forest | Brown soil | Sand and boulder clay |

Data come from the “Plans of Forest Management”, which are updated for each forest division every 10 years. The names of provenances correspond to the names of the forest divisions.
2.3. Experimental Design

Pots with seedlings were distributed in four randomly located plots. The dimensions of the tents and the plots in the open were 7.5 × 3.4 m. There were ten seedlings for each provenance and plot. In total, 240 seedlings were used in the experiment. Based on the earlier results obtained in the experiment with seedlings of the same species [5], two light treatments were established: high light (HL; 100% of full sun irradiance) and low light (LL; 40% of full irradiance). High light can be treated as the control. Our earlier results showed that in full irradiance, in non-limiting growth conditions, the apical annual increment of *A. alba* seedlings was reduced compared with the shade treatments. However, at the opposite of the general opinion about the high shade tolerance of *A. alba*, total seedling biomass increased in HL. In high shade (8% of full irradiance), seedlings responded typically for plants by the reduction of growth and photosynthesis compared with 40 and 100% of full irradiance [5]. Therefore, two treatments, HL and LL, were the most promising to address questions about the light requirements of *A. alba* and to obtain the new information about its physiological responses to light. Additionally, the treatment 40% of full irradiance is close to natural conditions under the canopy where seedlings of *A. alba* are planted. In the present experiment, two plots were situated in the open (HL) and two plots under a shading net transmitting 40% of full irradiance. The mean relative value of photosynthetic photon flux (PPF) was determined with two light sensors (Spectrum Technologies, USA) on sunny and cloudy days. Photosynthetic photon flux (400–700 nm, µmol m⁻² s⁻¹) was measured under shading net and in the open at the same time to calculate the relative PPF by means of the equation: 

\[
\text{relative PPF} = \sum_{n=1}^{120} \left( \frac{\text{PPF under shading net}}{\text{PPF in the open}} \right) \times 100,
\]

where \( n \) is the number of measurements under shading net and in the open.

2.4. Meteorological Conditions

In the open and in tents, air temperature and relative humidity were registered each hour with Hobo Pro v2 Temp/RH (Onset, Bourne, MA, USA). In the open, PPF was continually measured with the sensor (Apogee Instruments, Inc., Logan, UT, USA). Precipitation was monitored using the pluviometer coupled with the data logger Em50 (Decagon, Pullman, WA, USA). The volumetric substrate moisture in pots was occasionally controlled with 10HS sensor attached to ProChecker (Decagon) and continually monitored in pots in the open and in shade. All sensors used for continuous measurements were coupled with the data logger (Decagon). During the experiment, we did not observe any extreme climate conditions such as heat waves or extremely low precipitation.

2.5. Measurements of Growth

In April 2020, before bud opening, the diameter at the root collar (\( d_A \)) and the height (\( h_A \)) of all seedlings were measured. These measurements were repeated in June, after the bud set (\( d_J, h_J \)). The data were used to calculate the relative growth rate of \( d \) (RGR\(_d\)) and the relative growth rate of \( h \) (RGR\(_h\)) using the following equations: 

\[
\text{RGR}_d = \frac{d_J - d_A}{t_2 - t_1}, \quad \text{RGR}_h = \frac{h_J - h_A}{t_2 - t_1},
\]

where \( t_1 \) is the day of year in April and \( t_2 \) is the day of year in June on which growth measurements were conducted. In our experiment, \( t_2 - t_1 = 50 \) days.

2.6. Chlorophyll a Fluorescence

Chlorophyll a fluorescence was measured in needles of *A. alba* seedlings of six provenances acclimated to LL or HL using a fluorescence monitoring system (FMS 2, Hansatech, Norfolk, UK). This method of fluorescence measurements was previously described in detail in [31]. In brief, in August, at 9:00–9:30 a.m., five fully expanded current-year needles were collected from the last (youngest) whorl of five seedlings per light treatment and provenance, enclosed in Eppendorf tubes, and placed in a vacuum flask. In a laboratory, prior to fluorescence measurements, needles were dark-adapted for 30 min at air temperature ranging from 21 to 23 °C. Then, they were stuck on self-adhesive transparent tape to fill the entire aperture of the leaf clip. The mean air temperature of fluorescence
measurements was 22 °C (21–24 °C, min.–max.). The dark-adapted needles were exposed to modulated light at 0.05 µmol quanta m⁻² s⁻¹. After reading minimum fluorescence \(F_0\), a saturating 0.7 s pulse of light (PPF = 15.3 mmol m⁻² s⁻¹) was delivered to induce maximum fluorescence (\(F_m\)). The maximum quantum yield of PSII photochemistry was calculated as \(F_0/F_m\), where \(F_0 = F_m - F_0\). Next, needles in the clip were illuminated with increasing actinic light to generate light response curves of PSII quantum yield (\(\Phi_{PSII}\)). At each level, after a stable steady-state fluorescence (\(F_s\)) was reached, a 0.7 s saturating pulse was switched on and maximum light-adapted fluorescence (\(F'_m\)) was determined. At each actinic light level, the quantum yield of PSII was calculated with the equation \(\Phi_{PSII} = (F'_m - F_s)/F'_m\), and non-photochemical quenching of fluorescence (NPQ) was calculated as \(NPQ = (F_m - F'_m)/F'_m\) \cite{32}. The course of fluorescence was monitored online to ensure stable \(F_s\) values after changing actinic light levels before a saturating pulse was switched on. Usually, stabilization time of \(F_s\) took 1.0–3.5 min, depending on the level of actinic light. At each light level, the apparent rates of photosynthetic electron transport (ETR) were calculated as \(ETR = \alpha \times \Phi_{PSII} \times PPF \times 0.5\) (\(\alpha\)—needle absorptance, \cite{32,34}). Assumptions were made that the excitation energy is partitioned equally between the two photosystems (hence the factor of 0.5 \cite{32}). Leaf absorptance, however, differed among plant species and due to adaptation to the light environment. The parameter \(\alpha\) was calculated using total chlorophyll content in needles \cite{33}.

### 2.7. Light Curves of Chlorophyll a Fluorescence

The maximum apparent rate of photosynthetic electron transport of PSII (ETR\(_{max}\)) and the saturation level of photosynthetic photon flux density (PPF\(_{sat}\)) in Equation (1) were derived by fitting the functions of \cite{34}:

\[
ETR = \alpha \frac{1 - \beta PPF}{1 + \gamma PPF} PPF
\]

(1)

where \(\alpha\) is the initial slope, \(\beta\) is the extent of dynamic downregulation of PSII, and \(\gamma\) is defined as a saturation term of light response curve for photosynthetic electron transport rate (ETR-PPF); PPF is photosynthetic photon flux.

PPF\(_{sat}\) was calculated using Equation (2):

\[
PPF_{sat} = \frac{\sqrt{(\beta + \gamma)/\beta} - 1}{\gamma}
\]

(2)

ETR\(_{max}\) was defined as ETR at PPF\(_{sat}\), and was derived using Equation (3):

\[
ETR_{max} = \alpha \left(\frac{\sqrt{\beta + \gamma} - \sqrt{\beta}}{\gamma}\right)^2
\]

(3)

Quantum yield of PSII photochemistry and quantum yield of PSII photochemistry at saturating PPF (\(\Phi_{PPF_{sat}}\)) were calculated using Equations (4) and (5), as proposed by Ye et al. (in preparation):

\[
\Phi_{PSII} = \frac{\alpha \times \beta}{\alpha' \beta'} \times \frac{1 - \beta \times PPF}{1 + \gamma \times PPF}
\]

(4)

\[
\Phi_{PPF_{sat}} = \frac{\alpha \times \beta}{\alpha' \beta'} \times \frac{1 - \beta \times PPF_{sat}}{1 + \gamma \times PPF_{sat}}
\]

(5)

where \(\alpha'\) is the distribution coefficient of absorption light energy by PSII and PSI, which was assumed to be equal to 0.5 \cite{32,34}, \(\beta'\) is leaf absorptance and \(\alpha\) is the initial slope of the light response curve of the electron transport rate.

The PPF versus NPQ curves were fitted with the following exponential function:

\[
NPQ = m + a(1 - e^{(-bPPF)})
\]

(6)
Light curves of NPQ rise to infinity [32]. Therefore, NPQ was calculated at the arbitrary value of 345 μmol m$^{-2}$ s$^{-1}$ of actinic light (NPQ$_{345}$). Light curves of $\Phi_{PSII}$, ETR, and NPQ were fitted with the above functions using the nonlinear estimation of Levenberg–Marquardt in Statistica 13.1 (Tulsa, OK, USA).

2.8. Chemical Analyses of Photosynthetic Pigments

In August 2019 and 2020, needles from the current-year cohort (40–50 mg of fresh weight FW) were collected into Eppendorf tubes with moist paper. The needles used for chlorophyll and carotenoid content analyses were taken from the same one-year lateral stem increments as those for measurements of chlorophyll $a$ fluorescence. They were cut into 2 mm pieces and incubated in 5 mL 100% dimethylsulfoxide (DMSO) saturated with CaCO$_3$ to avoid pheophytisation at 60 °C in water bath until the solution became translucent (approximately 5 h). The absorbance of the extract was measured at 665, 648, and 470 nm. Chlorophyll $a$, $b$, and total carotenoids contents were calculated using the formulae given by Barnes et al. [35].

2.9. Needle Structure Measurement

Needle structure was determined using leaf mass-to-area ratio (LMA) (g m$^{-2}$). It was computed from oven-dry leaf biomass and needle projected area. Each needle sample was scanned (WinSeedle 2004, Regent Instruments Inc., Quebec, QC, Canada), dried (65 °C per 96 h) and weighed. A total of 5–10 needles per sample were used for LMA analyses.

2.10. Statistical Analysis

Prior to analyses, data were tested for normality and homogeneity. Growth and photochemical parameters were analyzed in a split-plot design where the main plot was the light effect and the subplot was the provenance effect [36]. An individual potted seedling was considered as the replication. The following model of split-plot design was applied: $Y_{ijk} = \mu + p_i + \tau_j + \delta_{ij} + \beta_k + (\tau\beta)_{jk} + \epsilon_{ijk}$, where $Y_{ijk}$ is the observation corresponding to $k_{th}$ level of sub-plot factor (provenance), $j_{th}$ level of main plot factor (light) and the $i_{th}$ replication; $\mu$ is the general mean; $p_i$ is the $i_{th}$ block (replication) effect; $\tau_j$ is the $j_{th}$ main plot treatment effect; $\delta_{ij}$ is the whole plot error; $\beta_k$ is the $k_{th}$ sub-plot treatment effect; $(\tau\beta)_{jk}$ is the interaction between $j_{th}$ level of main-plot treatment and the $k_{th}$ level of sub-plot treatment; and $\epsilon_{ijk}$ is the split-plot error. The error components $\delta_{ij}$ and $\epsilon_{ijk}$ are independently and normally distributed with means of zero and respective variances $\sigma^2_{\delta}$ and $\sigma^2_{\epsilon}$ [37]. When significant differences were found in ANOVA, an LSD test with Bonferroni modification was applied. Data which did not fulfill ANOVA conditions were analyzed using the nonparametric Kruskal–Wallis test ($F_v/F_m$). Ten potted seedlings per plot (replications), light treatment, and provenance were used for growth measurements, and five potted seedlings were used for photochemical parameters and photosynthetic pigments analyses. Photosynthetic pigment concentration was measured twice in 2019 and 2020; therefore, these data were analyzed with the split-split-plot design [36], where the main plot was time (year), the subplot was the light effect, and the sub-subplot was the provenance effect. At each light threshold in the light curves of $F_v/F_m$, $\Phi_{PSII}$, ETR$_{max}$, and NPQ$_{345}$, a t-test was performed to compare the mean values between LL and HL. Linear regression between structural needle parameters and between NPQ and ETR$_{max}$ was applied to show needle acclimation to the light environment. The provenances were grouped using hierarchical clustering. Complete linkage clustering, mean linkage clustering, and Ward’s minimum variance method were compared with the agglomerative coefficient ($AC$), and the method for which the value of $AC$ was closest to 1.0 was chosen to plot dendrograms. Needle structural and photosynthetic traits (ETR$_{max}$, Chl$_{tot}$, LMA), relative growth rates (RGR$_d$, RGR$_h$), and growth parameters ($d$, $h$) were used for clustering, respectively. All statistical analyses were conducted with R v. 3.6.3 [38].
3. Results

3.1. Growth Traits

In April, before bud opening, the effects of provenance and light x provenance interaction were significant for \( d_A \) and \( h_A \), but a light effect was not detected. At the end of June, seedlings differed significantly in \( d_J \) between the light treatment (\( HL 8.7 \pm 0.1, LL 7.5 \pm 0.1 \text{ mm} \)) and among the provenances, with \( S \) showing the lowest mean value of \( d_J \). The mean values of \( h_J \) differed among the provenances and these differences were modified by the light effect, and \( h_J \) did not significantly depend on the light treatment (Table 3, Figure 1a,b). Differences in \( d_J \) and \( h_J \) can be, at least to some extent, explained by the significant differences in \( RGR_d \) and \( RGR_h \) among the provenances (Table 3, Figure 1c,d). Provenances with the high \( RGR_d \) (\( B 0.064 \pm 0.004, M 0.062 \pm 0.004 \text{ mm day}^{-1} \)) were also characterized by high \( RGR_h \) values (Figure 1c,d). \( RGR_d \) was determined by the light environment (\( HL 0.070 \pm 0.002, LL 0.046 \pm 0.001 \text{ mm day}^{-1} \)), but \( RGR_h \), similarly to \( h_A \) and \( h_J \), was nonsignificantly higher in \( LL \) than in \( HL \) (\( HL 0.73 \pm 0.03, LL 0.81 \pm 0.04 \text{ mm day}^{-1} \)), supporting the opinion that \( A. \text{ alba} \) is highly shade-tolerant.

![Figure 1](image-url)

**Figure 1.** Growth parameters of *Abies alba* seedlings from six northern provenances measured in June 2019 (means ± SE). (a) Diameter at root collar in June \((d_J)\); (b) height of seedlings in June \((h_J)\); (c) relative growth rate at root collar \((RGR_d)\); (d) relative growth rate of height \((RGR_h)\). The results of ANOVA are shown in Table 3. The same letters above columns indicate that the mean values are not different using Tukey’s test at \( p < 0.05 \) (\( n = 40 \), number of seedlings per provenance). M—“Międzylesie”, KG—“Kamienna Góra”, S—“Syców”, K—“Kolumna”, B—“Brzeziny”, O—“Osusznica”.

Table 3. The split-plot analysis of variance for the effects of blocks (replicates), irradiance level (40% or 100% of full solar irradiance), and provenance of Abies alba seedlings on diameter at root collar and height of seedling measured in April and June 2019 ($d_A$, $d_J$, $h_A$, $h_J$) and on relative growth rates of diameter ($RGR_d$) and height ($RGR_h$) of silver fir seedlings originating from one of six provenances (see Table 1) and acclimated to full light or shade. The values of probability in bold indicate significant differences between means ($p < 0.05$). $Df$—degrees of freedom; $MS$—mean sum of squares; $F$—value of Snedecor’s function; $p$—probability.

| Trait | Effect     | Df | MS   | F   | p       |
|-------|------------|----|------|-----|---------|
| $d_A$ | Block      | 9  | 0.856| 0.59| 0.780   |
|       | Light      | 1  | 0.118| 0.08| 0.783   |
|       | Ea         | 9  | 1.459| 0.75| 0.861   |
|       | Prov       | 5  | 8.027| 24.53| <0.001 |
|       | Light × Prov| 5  | 1.242| 3.80| 0.004   |
|       | Eb         | 90 | 0.327| 0.75| 0.861   |
| $d_J$ | Block      | 9  | 1.280| 0.53| 0.823   |
|       | Light      | 1  | 85.419| 35.18| <0.001 |
|       | Ea         | 9  | 2.428| 0.73| 0.883   |
|       | Prov       | 5  | 5.430| 8.34| <0.001 |
|       | Light × Prov| 5  | 0.255| 0.39| 0.854   |
|       | Eb         | 90 | 0.653| 0.73| 0.884   |
| $h_A$ | Block      | 9  | 380.8| 0.19| 0.990   |
|       | Light      | 1  | 2287.8| 1.13| 0.317   |
|       | Ea         | 9  | 2033.7| 0.42| 0.999   |
|       | Prov       | 5  | 14,679.8| 27.30| <0.001 |
|       | Light × Prov| 5  | 4291.1| 7.98| <0.001 |
|       | Eb         | 90 | 537.7| 0.42| 0.999   |
| $\log h_J$ | Block | 9  | 0.002| 0.17| 0.993   |
|          | Light  | 1   | 0.0003| 0.03| 0.879   |
|          | Ea     | 9   | 0.013| 0.57| 0.998   |
|          | Prov   | 5   | 0.068| 17.82| <0.001 |
|          | Light × Prov| 5  | 0.015| 3.88| 0.003   |
|          | Eb     | 90  | 0.004| 0.57| 0.982   |
| $RGR_d$ | Block | 9  | 0.0001| 0.47| 0.861   |
|          | Light | 1   | 0.037| 37.16| <0.001 |
|          | Ea    | 9   | 0.001| 0.75| 0.866   |
|          | Prov  | 5   | 0.001| 5.04| <0.001 |
|          | Light × Prov| 5  | 0.0002| 0.84| 0.523   |
|          | Eb    | 90  | 0.0003| 0.75| 0.866   |
| $\log RGR_h$ | Block | 9  | 0.002| 0.11| 0.998   |
|            | Light | 1   | 0.018| 1.08| 0.326   |
|            | Ea    | 9   | 0.016| 1.09| 0.354   |
|            | Prov  | 5   | 0.025| 3.97| 0.003   |
|            | Light × Prov| 5  | 0.015| 2.41| 0.043   |
|            | Eb    | 90  | 0.006| 1.09| 0.354   |

3.2. Needle Structure and Photosynthetic Pigments

Year of sampling, light treatment, and seedling provenance significantly influenced needle structure, as determined using the leaf mass-to-area ratio ($LMA$ (g m$^{-2}$)) (Table 4). In HL, $LMA$ was higher than in LL ($164.6 ± 2.7$, $143.9 ± 2.6$, respectively). $S$ and $M$ showed the lowest values of $LMA$ ($144.4 ± 4.6$, $147.6 ± 4.9$), and needles of the other provenances had $LMA$ close to 159. Total chlorophyll content in needles depended on year of sampling, light treatment, and provenance of seedlings (Table 4). $Chl_{tot}$ (mg g$^{-1}$) was higher in shaded needles compared with those exposed to HL ($12.5 ± 0.5$, $9.2 ± 0.4$). The provenance effect on $Chl_{tot}$ (mg g$^{-1}$) and $Chl_{tot}$ (g m$^{-2}$) was significant, but the order of provenances depended on whether chlorophyll was recalculated by needle dry mass or a unit of needle area (Figure 2b,c). Carotenoid concentration [$Car$ (mg g$^{-1}$)], unexpectedly, was higher in
than in HL needles (1.96 ± 0.05, 1.70 ± 0.06), which suggested that their concentration was related to needle structure assessed with LMA. The differences in Car among the provenances were not significant (Table 4).

Table 4. The split-split-plot analysis of variance for the effects of blocks, date of sampling (year 2019, 2020), irradiance level (40% or 100% of full solar irradiance), and of provenance on leaf mass-to-area ratio and chlorophyll and carotenoid concentrations in needles of Abies alba seedlings. LMA—leaf mass-to-area ratio; Chl<sub>tot</sub>—total chlorophyll concentration; Car—carotenoid concentration. The values of probability in bold indicate significant differences between means (p < 0.05).

| Trait | Effect | Df | MS   | F     | p     |
|-------|--------|----|------|-------|-------|
|       | Block  | 4  | 292.9| 0.672 | 0.645 |
|       | Year   | 1  | 9322.8| 21.37 | 0.009 |
|       | Ea     | 4  | 436.2|       |       |
|       | Light  | 1  | 12,853.2| 15.73 | 0.004 |
|       | Year × Light | 1 | 1202.4| 1.471 | 0.260 |
|       | Eb     | 8  | 817  |       |       |
|       | Prov   | 5  | 858.5| 3.00  | 0.016 |
|       | Prov × Year | 5 | 167.5| 0.586 | 0.711 |
|       | Prov × Light | 5 | 242.5| 0.849 | 0.520 |
|       | Prov × Y × L | 5 | 156.3| 0.546 | 0.740 |
| LMA (g m<sup>−2</sup>) | Ec | 80 | 285.8|       |       |
|       | Block  | 4  | 0.97 | 0.414 | 0.793 |
|       | Year   | 1  | 343.9| 802.2 | <0.001 |
|       | Ea     | 4  | 2.3  |       |       |
|       | Light  | 1  | 322.8| 165.6 | <0.001 |
|       | Year × Light | 1 | 0.07 | 0.034 |       |
|       | Eb     | 8  | 1.95 |       |       |
|       | Prov   | 5  | 9.71 | 3.22  | 0.011 |
|       | Prov × Year | 5 | 4.30 | 1.43  | 0.224 |
|       | Prov × Light | 5 | 2.99 | 0.99  | 0.428 |
|       | Prov × Y × L | 5 | 2.77 | 0.92  | 0.472 |
|       | Ec     | 80 | 3.01 |       |       |
| Chl<sub>tot</sub> (mg g<sup>−1</sup>) |       |    |      |       |       |
|       | Block  | 4  | 0.052| 0.704 | 0.628 |
|       | Year   | 1  | 13.96| 188.83| <0.001 |
|       | Ea     | 4  | 0.074|       |       |
|       | Light  | 1  | 2.065| 15.15 | 0.005 |
|       | Year × Light | 1 | 0.525| 3.85  | 0.085 |
|       | Eb     | 8  | 0.136|       |       |
|       | Prov   | 5  | 0.114| 1.66  | 0.153 |
|       | Prov × Year | 5 | 0.069| 1.01  | 0.420 |
|       | Prov × Light | 5 | 0.169| 2.47  | 0.039 |
|       | Prov × Y × L | 5 | 0.213| 3.10  | 0.013 |
|       | Ec     | 80 | 0.069|       |       |
| Car (mg g<sup>−1</sup>) |       |    |      |       |
Figure 2. Structural needle traits of Abies alba seedlings from northern provenances (means ± SE). Data from 2019 and 2020 are pooled. (a) Leaf mass-to-area ratio (LMA); (b) total needle chlorophyll concentration (Chl_{tot} mg g^{-1}); (c) total needle chlorophyll content (Chl_{tot} g m^{-2}). The results of ANOVA are shown in Table 4. The same letters above columns indicate that the mean values are not different in Tukey’s test at \( p < 0.05 \) (\( n = 20 \), \( n - \) number of seedlings per provenance). M—“Międzylesie”, KG—“Kamienna Góra”, S—“Syców”, K—“Kolumna”, B—“Brzeziny”, O—“Osusznica”.

3.3. Photochemistry

\( \Phi_{PPF_{sat}} \) was not significantly changed by the light treatment or provenance (Table 5). \( ETR_{\text{max}} \) (\( \mu \text{mol m}^{-2} \text{s}^{-1} \)) was lower in needles of seedlings acclimated to LL compared with HL seedlings (110 ± 2, 134 ± 3). S showed the lowest mean value of \( ETR_{\text{max}} \) (115), but differed significantly only compared with M, and there were no significant differences
among the other provenances (Figure 3). NPQ$_{345}$, which is a measure of energy loss as heat, at actinic PPF = 345 µmol m$^{-2}$ s$^{-1}$ was higher in LL than in HL (2.51 ± 0.06, 1.81 ± 0.07). The significant interaction between the light treatment and provenance means that the light effect on NPQ$_{345}$ was modified by the provenance of seedlings (Table 5).

Table 5. The split-plot analysis of variance for the effects of blocks (replications), irradiance level (40% or 100% of full solar irradiance), and provenance of Abies alba seedlings on photochemical parameters: quantum yield of PSII photochemistry at saturating PPF ($\Phi_{PPFsat}$), maximal apparent electron transfer rate ($ETR_{max}$), and non-photochemical quenching of fluorescence at PPF = 345 µmol m$^{-2}$ s$^{-1}$ (NPQ$_{345}$). The values of probability in bold indicate significant differences between means ($p < 0.05$).

| Trait        | Effect   | Df | MS   | F     | p     |
|--------------|----------|----|------|-------|-------|
| $\Phi_{PPFsat}$ | Block    | 4  | 0.001 | 0.856 | 0.558 |
|              | Light    | 1  | 0.010 | 6.363 | 0.065 |
|              | Ea       | 4  | 0.002 | -     | -     |
|              | Prov     | 5  | 0.001 | 0.364 | 0.870 |
|              | Light × Prov | 5 | 0.005 | 1.678 | 0.162 |
|              | Eb       | 40 | 0.003 | -     | -     |
| $ETR_{max}$  | Block    | 4  | 618.6 | 1.339 | 0.392 |
|              | Light    | 1  | 12,604.7 | 27.28 | 0.006 |
|              | Ea       | 4  | 462.1 | -     | -     |
|              | Prov     | 5  | 505.0 | 2.703 | 0.034 |
|              | Light × Prov | 5 | 57.30 | 0.307 | 0.906 |
|              | Eb       | 40 | 186.8 | -     | -     |
| NPQ$_{345}$  | Block    | 4  | 0.510 | 1.844 | 0.284 |
|              | Light    | 1  | 7.504 | 27.12 | 0.006 |
|              | Ea       | 4  | 0.277 | -     | -     |
|              | Prov     | 5  | 0.081 | 1.220 | 0.318 |
|              | Light × Prov | 5 | 0.179 | 2.708 | 0.034 |
|              | Eb       | 40 | 0.066 | -     | -     |

Figure 3. Maximal electron transfer rate (mean ± SE) of Abies alba seedlings originating from different provenances. The results of ANOVA are shown in Table 5. The same letters above columns indicate that the mean values of $ETR_{max}$ do not differ significantly among the provenances in a posteriori Tukey’s test at $p < 0.05$ ($n = 10$). The provenances are ranged from south to north: M—“Międzylesie”, KG—“Kamienna Góra”, S—“Syców”, K—“Kolumna”, B—“Brzeziny”, O—“Osusznica”. 

Figure 3. Maximal electron transfer rate (mean ± SE) of Abies alba seedlings originating from different provenances. The results of ANOVA are shown in Table 5. The same letters above columns indicate that the mean values of $ETR_{max}$ do not differ significantly among the provenances in a posteriori Tukey’s test at $p < 0.05$ ($n = 10$). The provenances are ranged from south to north: M—“Międzylesie”, KG—“Kamienna Góra”, S—“Syców”, K—“Kolumna”, B—“Brzeziny”, O—“Osusznica”.
The light curves of ETR showed three steps: (1) an increase with actinic PPF, where the curves from both light treatments overlapped; (2) the saturation level of ETR, which was lower in LL than in HL; (3) the decrease in ETR with greater actinic light above PPF\textsubscript{sat} due to PSII downregulation (Figure 4a–f). In each provenance, curves from LL and HL diverged at the actinic PPF ≈ 530 \(\mu\)mol m\(^{-2}\) s\(^{-1}\); however, the provenance O, in contrast to the other provenances, did not show significant differences between the mean values ETR at each actinic light level in LL and HL (Figure 4f). \(\Phi_{\text{PSII}}\) decreased with increasing actinic light level more abruptly in LL than in HL (Figure 5a–f). Compared with the other provenances, in M and O there were no significant differences in \(\Phi_{\text{PSII}}\) (Figure 5a,f). Light curves of NPQ increased with increasing actinic light values without attaining a saturation level (Figure 6a–f). When the mean values of NPQ were compared between the light treatments in each provenance separately, significant differences were found, except for O (Figure 6f). These differences were detected at the values of PPF 208, 345, 530, 783, and 1075 in S.

![Figure 4](image_url). Light curves of apparent electron transfer rate (ETR) in needles of Abies alba seedlings originating from different provenances and acclimated to full irradiance or shade. Mean values (±SE) of ETR are shown separately for each provenance in plots (a–f) in shade and in full irradiance. An asterisk indicates that mean values of ETR at the given actinic PPF value are significantly different using Student’s t-test at \(p < 0.05\) (\(n = 5\)). PPF—photosynthetic photon flux.
Figure 5. Light curves of PSII quantum yield photochemistry ($\Phi_{PSII}$) in needles of *Abies alba* seedlings originating from different provenances and acclimated to full irradiance or shade. Mean values (±SE) of $\Phi_{PSII}$ are shown separately for each provenance in plots (a–f) in shade and in full irradiance. An asterisk indicates that mean values of $\Phi_{PSII}$ at the given actinic PPF value are significantly different using Student’s *t*-test at $p < 0.05$ ($n = 5$). PPF – photosynthetic photon flux.
Figure 6. Light curves of non-photochemical quenching of fluorescence (NPQ) in needles of *Abies alba* seedlings originating from different provenances and acclimated to full irradiance or shade. Mean values (±SE) of NPQ are shown for each provenance in plots (a–f) in shade and in full irradiance. An asterisk indicates that the mean values of NPQ at the given actinic PPF value are significantly different using Student’s t-test at $p < 0.05$ ($n = 5$). PPF — photosynthetic photon flux.
3.4. Correlations with Climate of Seed Stands and Cluster Analysis

Pearson’s coefficients of correlations between the values of climatic characteristics of the mother stands and the mean values of seedlings’ physiological parameters were significant in LL at 0.01 ≤ p < 0.05 (Table 6). The mean warmest month temperature (T_{max}) was positively related to Chl_{tot} (mg g^{-1}) and chlorophyll a/b ratio. Chl_{tot} increased with TD (continentiality) and mean summer heat:moisture index (SH). NPQ_{345} increased with MCTM. RGR_{d} and Chl_{tot} (g m^{-2}) decreased with mean annual precipitation (P) and increased with AHM.

Table 6. The relationships between the climate characteristics of the mother stands and mean values per provenance of growth and leaf traits. Pearson’s coefficients of correlation (r), together with the values of probabilities, are given.

| Climate Parameter | RGR_{d} | NPQ_{345} | Chl_{tot} (mg g^{-1}) | Chl_{tot} (g m^{-2}) | al/b |
|-------------------|---------|-----------|----------------------|----------------------|------|
| T_{max}           | -       | -         | 0.889 *              | -                    | 0.834 *|
| T_{min}           | -       | 0.874 *   | -                    | -                    | -    |
| TD                | -       | -         | 0.903 *              | -                    | -    |
| P                 | -0.895 *| -         | -                    | -0.878 *             | -    |
| AHM               | 0.840 * | -         | -                    | 0.853 *              | -    |
| SH                | -       | -         | 0.848 *              | -                    | -    |

The correlations were significant for the low light treatment (* 0.01 ≤ p < 0.05). Mean values of measured parameters were calculated for each provenance, and they were correlated with the climatic variables (n = 6, n—number of pairs of values). Mean values of relative growth rates at root collar (RGR_{d}) were calculated for 20 seedlings per provenance, means of non-photochemical quenching of fluorescence at actinic light 345 μmol m^{-2} s^{-1} (NPQ_{345}), and means of total chlorophyll concentration per g needle dry mass or per m^{2} needle area (Chl_{tot}) for 5 or 10 seedlings per provenance, respectively. T_{max}—mean warmest month temperature (°C); T_{min}—mean coldest month temperature (°C); TD—temperature difference between T_{max} and T_{min}, or continentality (°C); P—mean annual sum of precipitation (mm); AHM—mean annual heat:moisture index (T_{mean} + 10)/(P/1000); SH—mean summer heat:moisture index [(T_{max})/(P_{s}/1000)].

For cluster analyses, the traits showing highly significant differences among provenances were chosen. The agglomeration coefficient value of 0.9 indicated that RGR_{d} and RGR_{h} better discriminated the clusters of the study provenances than structural and physiological leaf traits (LMA, Chl_{tot} (mg g^{-1}) and ETR_{max}), which showed an agglomeration coefficient 0.6 (Figures 7 and 8). When RGR_{d} and RGR_{h} were used for cluster analysis using Ward’s method, three groups of provenances were distinguished: B—M, KG—O, and K—S. The complete linkage clustering for LMA, Chl_{tot} (mg g^{-1}), and ETR_{max} also made it possible to distinguish three different clusters of provenances: M—S, B—O, and KG—K (Figure 8).

Figure 7. Dendrogram of cluster analysis using complete linkage clustering for needle traits: leaf mass-to-area, total chlorophyll concentration, and maximal electron transfer rate (LMA, Chl_{tot}, ETR_{max}). Agglomeration coefficient = 0.6.
4. Discussion

4.1. Acclimation to Light

The *Abies alba* seedlings used in our study acclimated to the contrasting light environments of LL (40% of full irradiance) or HL (100%) in terms of growth, photosynthetic pigment concentration, and needle photochemistry. The results support the hypothesis that under non-limiting water and nutrient conditions, light is the main driver of growth, needle structure, and photochemical processes in *A. alba*. The mean value of diameter at the root collar was higher in HL than in LL, but the height of seedlings did not differ between the light treatments. The differences in $d_1$ were due to the higher $RGR_d$ in HL compared to LL. The relative growth rate of height was not changed by the light environment. This is consistent with our earlier results in controlled conditions, which showed that at the age of 4–5 years, an increase in light level above 18% was not beneficial for the annual height increment of *A. alba* seedlings [5]. When the values of growth parameters in different light environments are compared among different controlled and forest experiments, it is evident that during the juvenile stage of development, the high shade tolerance of *A. alba* results in the ability to grow slowly in high shade when limiting the increase in the annual height increment at mid (40%) and high light (100%) [5,39,40].

In our study, LMA was higher in HL compared with LL needles, which is in accordance with earlier studies in controlled conditions and in forest [5,6]. Leaf mass-to-area ratio typically increased with higher irradiance due to the greater thickness and tissue density in HL than in LL [41]. In contrast to our present and earlier results, in the western Spanish Pyrenees, no differences were found in LMA, needle anatomical and ultrastructural traits, or gas exchange parameters when understory *A. alba* trees were compared with trees growing in the open [42]. When compared with our present study, these contradictory results can probably be attributed to the lower plasticity of the rear-edge Spanish provenance of *A. alba* compared with the higher plasticity of the northern provenances in response to light; however, this observation should be verified with rear- and leading-edge populations under common experimental conditions [43].

Total chlorophyll and carotenoid concentrations were higher in LL than in HL, indicating that concentrations of these two groups of pigments were positively correlated, and in non-limiting experimental conditions, HL did not induce a photoprotective increase in *Car* [44–46]. It can be suggested that in our study, the decrease in both *Chl* and *Car* might reflect an acclimation to HL and play a photoprotective role [45]. This is in agreement with the earlier study showing that in winter and in spring, under high light and low temperatures, *Chl* was reduced in needles of *A. alba*, *P. sylvestris*, *Picea abies*, and *Pinus mugo* Turra [47]. This result suggests that the opinion about the high shade tolerance of *A. alba* cannot exclude that under non-limiting growth environments, this species is able to adapt the photosynthetic apparatus to HL. In natural conditions, however, *A. alba* is sensitive to low temperatures in winter and spring frosts, which, together with high light, induce PSII downregulation or photoinhibition in the open [47].
In our experiment, the high plasticity of the *A. alba* photosynthetic apparatus was reflected by the acclimation of photochemistry to LL and HL. The higher values of \( ETR_{\text{max}} \) and \( NPQ_{345} \) and the course of \( ETR, \Phi_{\text{PSII}} \), and \( NPQ \) light curves in HL compared with those in LL indicated that this extremely shade-tolerant species may cope with full light in a non-stressed environment. In natural conditions, however, the additional stress of low temperature or drought together with high light can damage proteins involved in photochemistry and transiently or permanently reduce photochemical capacity [48–50].

### 4.2. Provenance Effect

Although the geographical distances among our study provenances were small, variations in ecophysiological traits among progenies representing the *A. alba* populations originating from the northern edge of its natural range indicated that some of them might represent different ecotypes. This was supported by among-provenance differentiation of growth, photosynthetic pigments, and \( ETR_{\text{max}} \). Compared with the other study provenances, \( S \) was distinguished in terms of \( d_j \) and \( h_j \), partly due to the lower \( RGR_d \) and \( RGR_h \), although the climate environment of our common garden was more similar to \( S \) compared with the montane provenances or with \( O \). Additionally, before the experiment with light treatments, we observed the high inter-provenance variation with seed stratification time (P. Robakowski, unpublished data). \( S \) seeds were characterized by an unusually long stratification time of 5 months, compared with 3 months for \( M, K \), and \( B \), and 2 months for \( O \). The longer stratification time and later germination affected the initial growth dynamics of \( S \) seedlings compared with the other study provenances. Inter-provenance differences in \( RGR \) found in our study have practical importance not only because higher \( RGRs \) mean higher biomass production, but also because of lower threat due to ground frosts and frost pockets at young age [14]. In long-lasting provenance trials, however, growth dynamics may vary depending on provenance, age of trees, and on current site conditions.

Variation in the values of \( Chl_{\text{tot}} \) suggested that our study provenances manifested differences in their ability to absorb light and to use it for photosynthesis; however, these inter-provenance differences depended also on needle structure (LMA) (Figure 2). The provenance \( O \), originating from the outside of the natural geographic range of *A. alba*, showed the highest \( Chl_{\text{tot}} \) (\( g \text{ m}^{-2} \)). Interestingly, when the light curves of \( ETR, \Phi_{\text{PSII}}, \) and \( NPQ \) were compared between the light treatments within each provenance, the mean values of these parameters were higher in HL than in LL; however, only in \( O \) were these differences not significant for all photochemical parameters. The cluster analysis indicated that \( O \) is similar to \( B \) with respect to \( RGR \) (Figure 7), but also to the Sudety Mountains provenance \( K \) in terms of physiological parameters (Figure 8). This is consistent with the results of genetic marker analyses in mitochondrial and chloroplast DNA, which provided evidence that the Sudety Mountains are the most probable region of origin for the *A. alba* provenance “Osusznica” (\( O \)) [30].

We found the relationships between the mean values of \( RGR_d, NPQ_{345}, \text{CHL}_{\text{tot}}, \) and some climatic parameters and indexes of their origins. \( Chl_{\text{tot}} \) increased with \( T_{\text{mmax}}, TD, \) and \( SH \). Losses of energy as heat increased with decreasing \( T_{\text{mmin}}, \) which suggested that provenances adapted to lower temperatures were able to dissipate excessive energy more efficiently than those growing at higher \( T_{\text{mmin}} \). The values of mean annual heat moisture index were positively correlated with \( RGR_d \), also suggesting local adaptation of the study populations to air temperature and precipitation. These correlations, however, have to be interpreted with caution, because of the low number of provenances used for the analyses. In comparison to our study, Konôpková et al. [18] found significant relationships between the thermostability of PSII and some climatic parameters in the provenance trials with 17 provenances. When six provenances from different altitudes were compared, significant climatic clines were found between the photosynthetic performance, altitude, and precipitation at the site of origin [51]. Our results are consistent with these earlier findings and additionally provide evidence that intraspecific variation in *A. alba* may be significant on small geographical scales.
4.3. Importance of Phenotypic and Genetic Variation for Abies alba Restoration

The value of using native tree species such as A. alba in ecosystem restoration is receiving growing recognition among practitioners; however, insufficient attention has been given to physiological and genetic variation within species [52]. Our results provide new insight into phenotypic intraspecific diversity of A. alba, which could be potentially important for resilience of forests with a greater share of this keystone species, in response to different stressors and climate changes. Ecotypic variability of A. alba at the northern edge of its natural range is instrumental to pursuing the program of this species’ restoration in the Polish Sudety Mountains and pertinent to the conservation of its local genetic diversity [26].

5. Conclusions

Our results indicate that during the juvenile stage of development, the high shade tolerance of A. alba results in the ability to grow slowly in high shade when limiting the increase in the annual height increment at mid and high light. Inter-provenience differences in RGR found in our study have practical importance because higher RGRs mean higher biomass production and lower threat due to ground frosts and frost pockets at young age. In this study, the decrease in photosynthetic pigments concentration and the higher needle photochemical performance reflected an acclimation to HL and played a photoprotective role. An extremely shade-tolerant A. alba coped with full light in a non-stressed environment. In natural conditions, however, A. alba may suffer from low winter temperatures, spring frosts, and high light which act in concert, inducing photoinhibition. Although the geographical distances among our study provenances were small, variations in ecophysiological traits among progenies and their correlations with the climate conditions of the origins suggested that some of them represented different ecotypes, which might differ in usefulness for the A. alba restoration under global climate changes.

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