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Research Article

Responses of silver fir (*Abies alba* Mill.) seedlings from different geographical locations to low temperature stress

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Abstract

Climate change has exacerbated difficulties for both the environment and humans in recent years, with major consequences on the resilience and ecological diversity of forests, including those of silver fir (*Abies alba* Mill.). Because cold stress is an important challenge to silver fir seedling growth, particularly in the early phases of development, the goal of this study was to find potential genetic resources with appropriate responses to the action of low temperatures. Thus, traits of interest were studied in the early stage of seedlings from seven different Romanian provenances. Soil electrical conductivity, root weight, and total seedling weight were found to be related to seedling growth and biomass elements. The results revealed substantial differences depending on geographical origin. Garda Seaca provenance had the highest seedlings tolerance. Exposure to varying low temperatures revealed minor variations between seedlings from the control and those treated to −20 °C, which might be explained by the current temperatures in Romania's silver fir producing area. However, exposing to −40 °C all seedlings showed deterioration. Soil electroconductivity reduced as exposure temperature decreased, emphasizing the link between cold stress and soil effects on fir seedling growth. Some Romanian provenances could be useful for future silver fir breeding or afforestation and reforestation operations.

Keywords: abiotic stress; climate change; cold stress; coniferous; silver fir

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Introduction

Scientists and society have become increasingly concerned about climate change in recent years. Numerous abiotic stressors, such as soil salinity, temperature and drought, influence plant and agricultural growth, as well as the distribution of wild species in the natural environment (Kopecká *et al.*, 2023). Furthermore, temperature stress limits seedlings' growth, especially in the early stages of plant development. Taking into account all these, coniferous forests are considered to be susceptible ecosystems to the effects of climate change (Leites *et al.*, 2023). Climate change is anticipated to have huge effects on forests globally. The world's forests are among our most prominent natural resources, giving immeasurable ecological, social and economic services. They are under greater pressure than at any time in the past and face the consequences of human population growth, progress in economic services and increasing climate change (Trivedi, 2023; Sahoo *et al.*, 2023).

In general, abiotic factors can affect terrestrial ecosystems by both decreasing and increasing above certain limits the optimal values for plants, acting as ecological limiting factors on certain plant communities (Miron *et al.*, 2018). Water, along with solar radiation, are essential ecological factors for life on Earth. Temperature is also one of the most important ecological factors limiting plant bio-productivity (Zhang *et al.*, 2005). It is also a major environmental factor that limits the distribution, productivity, and survivability of plants (Theocharis *et al.*, 2012; Wu *et al.* 2015). One of the basic processes governing productivity and growth of silver fir, that may be severely affected by temperature, is carbon gain (Robakowski *et al.*, 2002). Even more, the association of soil water deficit with thermal stress can drastically and irreversibly affect vegetative and reproductive development of plants (Zhang *et al.*, 2020).

Silver fir (*Abies alba* Mill.) is one of the most important forest trees in Central Europe, particularly in mountainous terrain and in the continent's southern and eastern regions (Ancuceanu *et al.*, 2023). It is acknowledged as a valuable forest species due to its numerous uses (Todea *et al.*, 2019; Todea *et al.*, 2020). However, managing its genetic resources and effectively conserving such populations, faces tremendous challenges in the current state of climate change and the applied forestry strategies, while germination of the species is known to be problematic.

Recently a clear decline of *A. alba* stands in Central European forests was reported (Novák and Kacálek, 2023). This is concluded to be determined not by a single inducing factor, but reflecting a more concerning behaviour of the species, and more specifically, its low resistance and low adaptability to different adverse environmental factors, that appear now days even within its natural distribution areas, and represent limitative circumstances.

Though silver fir, as a species, has a rather wide ecological distribution, it is possible that phenomena triggered by the global changes have irreversible effects on *Abies* populations (Slugeňová *et al.*, 2011). The current study adds new data regarding the variability and adaptability of the species and contributes to the finding of possible genetic resources with an adequate response to the effects of various abiotic stressors, considering the fact that in Romania, fir and spruce are the most important coniferous species, both from economic and ecological perspectives.

The main purpose of this study was to identify possible genetic resources with an adequate response to the action of low temperatures, considering cold as one of the abiotic stress factors. Secondly, electric conductivity of the soil was correlated with the growth parameters of the tested seedlings originated from different areas (provenances).

Materials and Methods

Seedlings' origin, growth conditions and stress treatments

A. alba Mill. seedlings were obtained from different areas in Romania, from seed stands included in the National Catalogue of Forest Genetic Resources and Forest Reproductive Materials. The geographic locations are descripted of each population in Table 1 and represented in Figure 1. All the provenances originated in areas of the Romanian Carpathians, five of them being located in the north-western part of the Romania, one in the northern part and one in the central part.

Thus, one-year-old seedlings were collected and stored at a constant temperature of 20 °C for several days in ICHAT laboratory (USAMV Cluj-Napoca) in order to perform the related measurements and analyses. Seedlings were maintained in 0.3-liter individual pots containing Humin-substrate N3 (Klasmann-Deilmann, Germany), in plastic trays, with controlled temperature and under natural light.

To test the performance of the seedlings to various low temperatures, three thermal stress treatments at −20 °C, −30 °C and −40 °C respectively were started, after 14 days of laboratory acclimatization as stated above, using different freezers. There were also taken care in parallel of seedlings for each provenance, kept in normal laboratory conditions, considered as control. After 30 days, samples were collected for each provenance, for each treatment, in order to measure a set of growth parameters: seedlings diameter, height, number of needles, root weight and total weight of the seedlings.

Substrate samples were also taken from the pots, to test the electrical conductivity, as detailed further.

Table 1. The studied *A. alba* provenances with specific characteristics

Nr	Population	County	Administrative location	Latitude/ Longitude	Mean annual temperature (°C)	Mean annual precipitation (mm)	Altitude (m asl)
1.	Valea Bistrei	Alba	OS. Valea Ariesului, UP II, u.a. 228B	46°27' N / 23°01' E	5.5	922	1050 - 1325
2.	Someșul Rece	Cluj	OS. Someșul Rece UP I u.a. 92A	46°38' N / 23°14' E	7.1	885	690 - 1250
3.	Avrig	Sibiu	OS Izvorul Florii UP III u.a 75A	45°37' N / 24°27' E	5.7	798	900 - 1150
4.	Budescu	Maramureș	OS. Poieni, UP IV, u.a 96 A	47°54' N / 24°36' E	6.2	986	860 - 1120
5.	Sohodol	Alba	OSP. Abrud UP IV 18 C	46°20' N / 23°06' E	5.3	794	870 - 1030
6.	Valea Morii	Alba	OSP. Abrud U V, u.a 39	46°19' N / 22°56' E	5.1	850	1080 - 982
7.	Gârda Seacă	Alba	OS. Gârda UP VI, u.a 20 H	46°31' N / 22°46' E	4.9	822	1090 - 1285

Note: Populations and description code (administrative location) are detailed according to the National Catalogue of Forest Genetic Resources, Bucharest 2011.

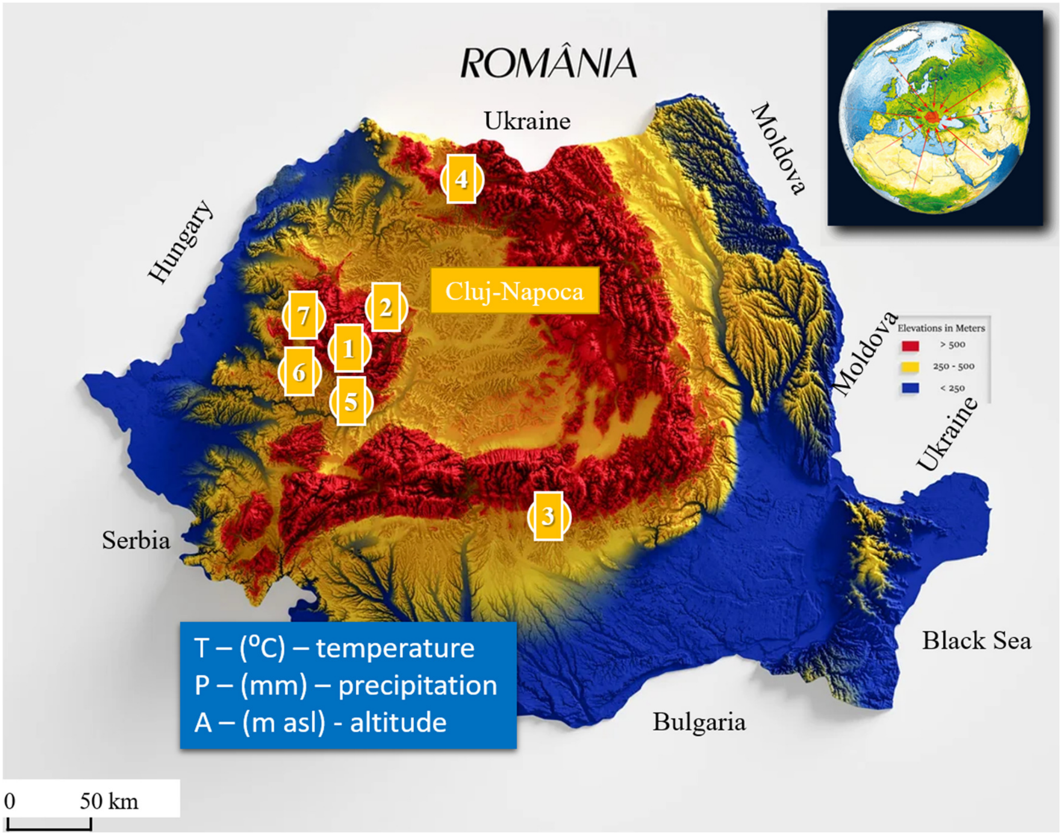

Figure 1. Geographical location of the analysed provenances of *A. alba*
Note: 1. T - 5.5; P - 922; A - 1050-1325; 2. T - 7.1; P - 885; A - 690-1250; 3. T - 5.7; P - 798; A - 900-1150; 4. T - 7.2; P - 986; A - 860-1120; 5. T - 5.3; P - 794; A - 870-1030; 6. T - 5.1; P - 850; A - 1080-982; 7. T - 4.9; P - 822; A - 1090-128

Substrate analysis

At the end of the thermal treatments, electrical conductivity was determined in the soil samples. The electrical conductivity (EC) is a parameter related to the concentration of ions in the soil and, therefore, to the degree of abiotic stress affecting the plants (Bresler *et al.*, 1982). Electrical conductivity (EC1:5) was measured with a conductivity-meter and expressed in dS $^{m-1}$.

Analysis of plant growth parameters

Before starting the stress treatments (time 0), the stem length and the number of needles were determined for all *A. alba* seedlings. To analyse the effects of cold stress at different negative temperatures, in the stage of early vegetative growth, the following parameters were determined also at the end of the treatments (time 1), in control and stressed seedlings: increases in stem length (SL) and in the number of needles (Nno) with respect to the values measured at time 0; root weight (RW) and total weight (TW) of the plants (Figure 2). The precision balance was used to determine the weight mass, and a specialized software (Digimizer Image Analysis Software) was used for to measure the length.

Figure 2. *A. alba* seedlings measurements realised in laboratory before and after the thermal stress treatments

Statistical analyses

Data were analysed using the program Statgraphics Centurion XVI (Statgraphics Technologies, The Plains, VA, USA). Significant differences between treatments were tested by one-way analysis of variance (ANOVA) at the 95% confidence level, and post hoc comparisons were made using the Tukey's HSD test at $p < 0.05$. Hierarchical cluster analysis (HCA) and the corresponding heatmap were performed using the ClustVis tool (Metsalu and Vilo, 2015) for traits for which significant differences ($p < 0.05$) had been detected in the ANOVA, with the purpose to find a signature of responses specific to the abiotic (thermal) stresses applied.

Results

Plant growth analysis and the substrate analysis

Seedlings collected from different provenances, with distinct ecological parameters, display a strong variability for means of number of needles, length and weight.

Regarding the seedling's length, Gârda Seacă provenance recorded the highest value for all treatments (with 8.66 cm for the −40 °C treatment and 10.14 cm for the control) (Figure 3).

Considering the number of needles, it was noticed a significant decrease as the temperature dropped. It is important to mention that cold stress exposure at various low temperatures revealed no differences between seedlings from different geographic origins at −20 °C, thus at this temperature, most probably expected in the species natural areal, the studied trait was not affected.

Cold stress exposure at various low temperatures revealed no differences between height, diameter, and number of needles within the seedlings from different geographic origins, when exposed at −20 °C, but seedlings exposed to −40 °C were compromised within all provenances investigated.

Regarding the seedlings diameter for all the treatments significant differences were noticed between provenances (Figure 4). What is worth to mention is that between the control and −20 °C exposure, the differences were not significant, showing once again that a stress of −20 °C would not affect this species. Instead, a decrease in values can be noticed as the temperature decreases, so that at −40 °C the lowest values were recorded. Even so, among the populations, at −40 °C, the highest values for the diameter were recorded in Avrig followed by Valea Bistrei.

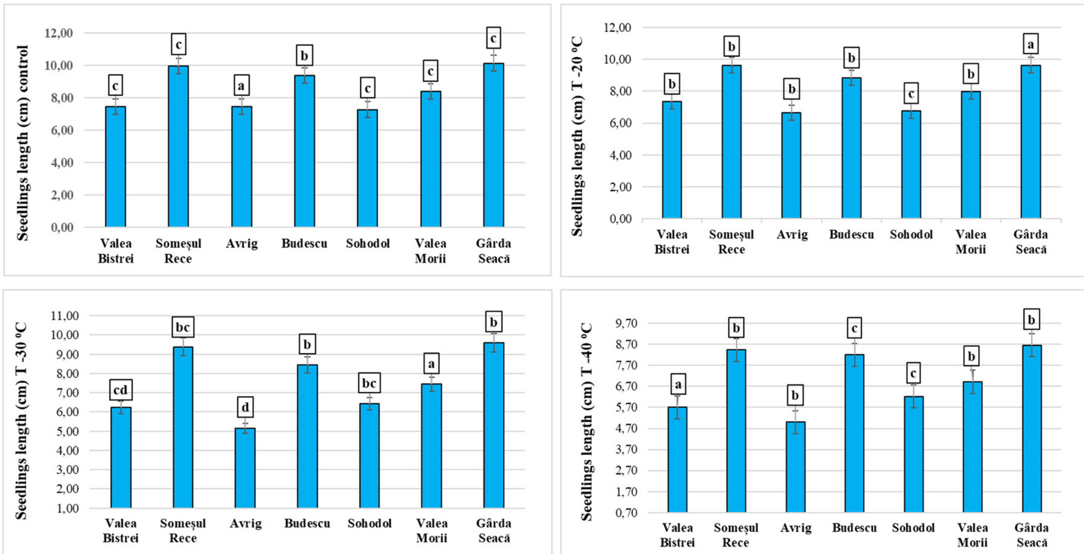

Figure 3. Effect of 30 days of thermal stress treatments on stem elongation in *Abies alba* seedlings
Bars represent mean values ± SD. Different lowercase letters within the bars indicate significant differences between origins according to the Tukey post-hoc test ($p < 0.05$)

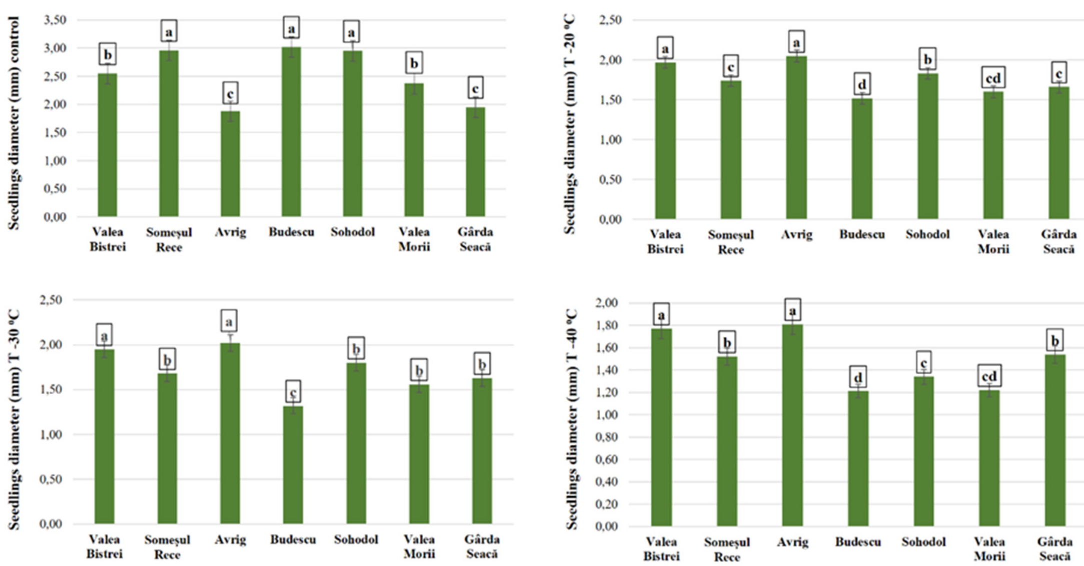

Figure 4. Effect of 30 days of thermal stress treatments on stem diameter in *Abies alba* seedlings
Bars represent mean values ± SD. Different lowercase letters within the bars indicate significant differences between origins according to the Tukey post-hoc test ($p < 0.05$)

The results of EC revealed significant differences between thermal treatments (Figure 5). The EC of the soil decreased as the temperature dropped. It was noticed that for the treatment with −20 °C no significant differences were recorded between the all the provenances, but at −30 °C and especially at −40 °C significant differences appeared. For the lowest temperature −40 °C, EC of the soil, the highest value was recorded at the Valea Morii and Garda Seaca provenances, and the lowest at Budescu provenance.

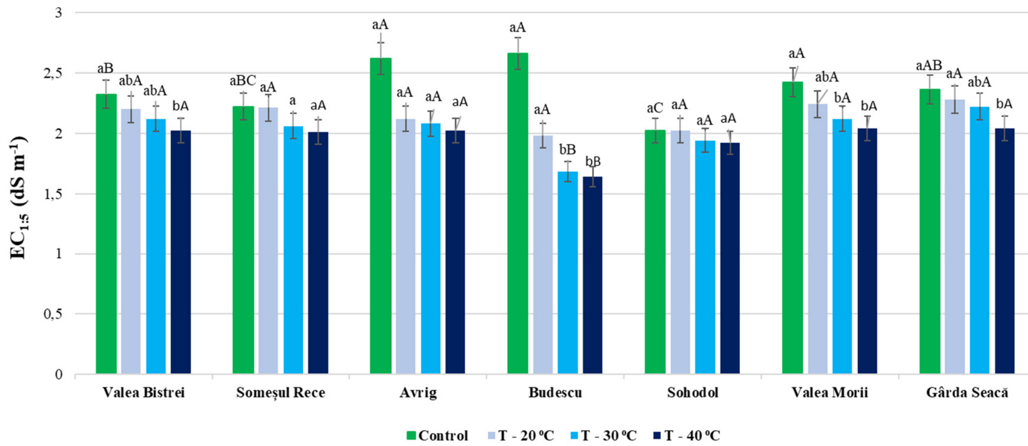

Figure 5. Substrate electrical conductivity (EC1:5) in the control seedlings and after 30 days of thermal stress at different low temperatures

Different lowercase letters above the bars indicate significant differences between treatments for each population, and different capital letters indicate significant differences between populations for plants undergoing the same treatment, according to the Tukey test ($p < 0.05$)

Analysing root weight, hierarchical cluster illustrated an appropriate linkage between the −30 °C and −40 °C treatments, as well as a grouping between the seedlings in the control and those subjected to thermal stress at −20 °C (Figure 6). It was also notable a grouping of the seven sources analysed, thus, Gârda Seacă formed a single main cluster, while the rest of the provenances formed a second main cluster. As noted in the official registration data (Table 1), the mean temperature at this area is the lowest within the investigated provenances, even if only small differences are noted among some provenances closely located, so that is may be assumed that the seedlings originated from the respective provenance might be more adapted to low temperatures.

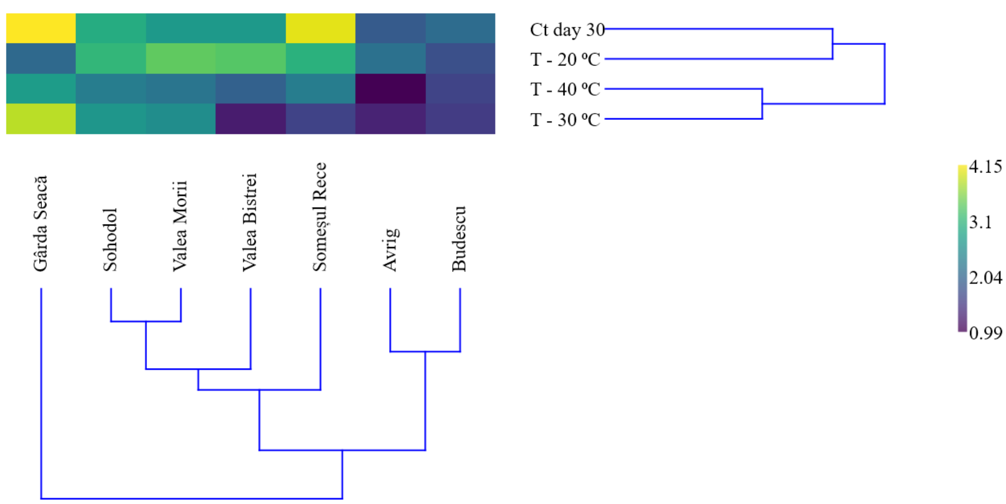

Figure 6. Hierarchical cluster in regard with the root weight of the seedlings from seven Romanian provenances of *A. alba*, subjected to 30 days of thermal stress treatments

Regarding the total weight of the seedlings, it was noticed a grouping of the seedlings depending on the applied treatments, in which two main clusters were similarly formed: one made up of the treatments −30 °C and −40 °C, and the other from the control seedlings and −20 °C, illustrating a connection between these. Also, two major clusters were formed in terms of the proximity between the source. Avrig and Valea Morii formed a cluster, and the other sources formed the second cluster (Figure 7).

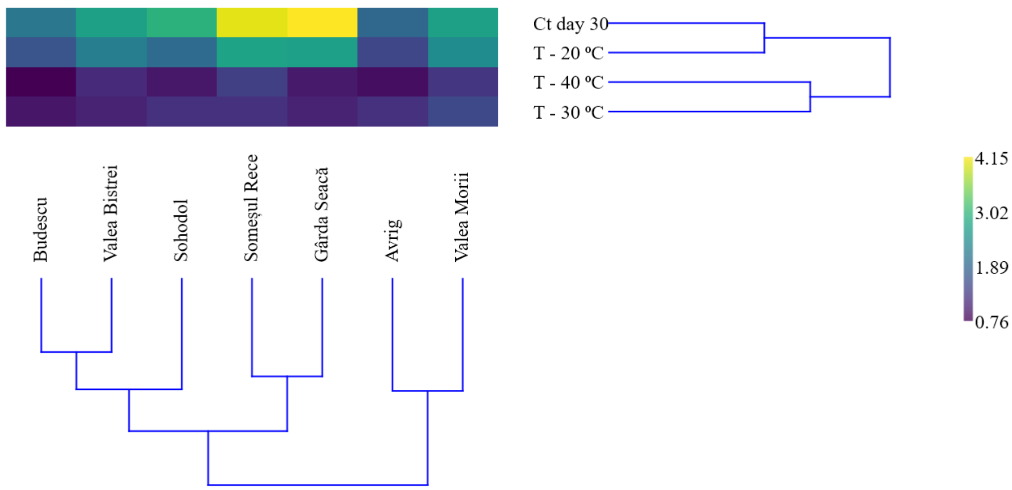

Figure 7. Hierarchical cluster in regard with the total weight of the seedlings from seven Romanian provenances of *A. alba*, subjected to 30 days of thermal stress treatments

Principal Component Analysis (PCA) is a statistical technique used to analyze and simplify data sets by transforming them into a set of linearly uncorrelated variables called principal components. In our experimental conditions PCA showed a clear separation of the seedlings response when considering both the provenance of origin and thermal stress treatments (Figure 8). This indicates differences in the growth rates of the seedlings from different provenances and showed that the different genotypes do not respond in the same way to stress conditions, at least not in our experimental conditions.

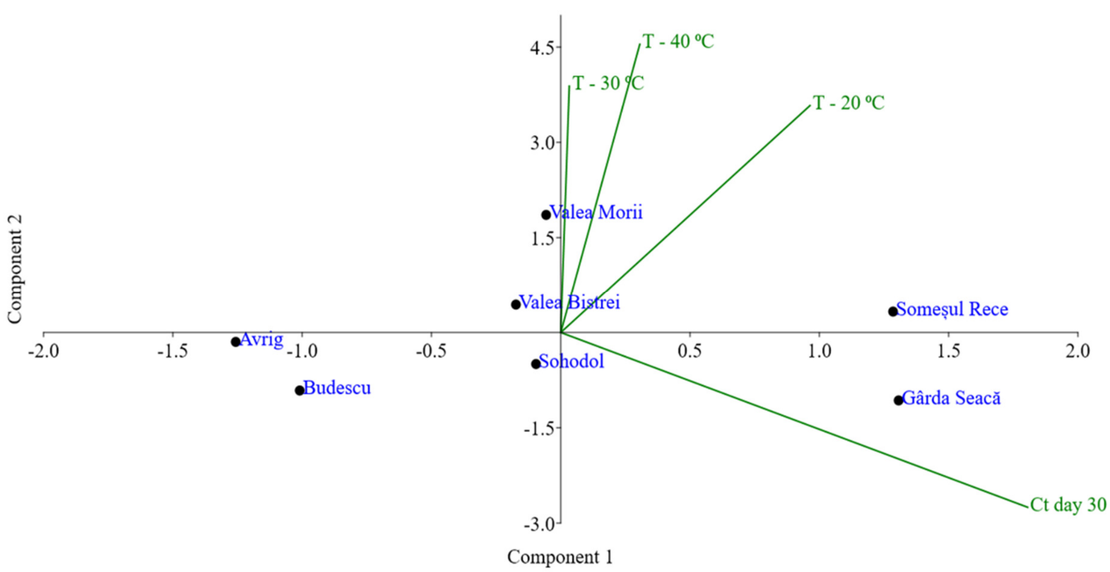

Figure 8. Principal Component Analysis (PCA) based on the first and second components, observed in seven Romanian provenances of *A. alba* subjected to one-month thermal stress treatments

Statistics analysis of data

The ANOVA highlighted the effect of the thermal stress treatments and the populations, as well as their interaction on the analyzed parameters compared to the controls. All analysed parameters showed a significant variation according to 'treatment'. According to the 'population' variable, all other parameters showed significant differences between populations Garda Seaca being the one that significantly differentiated itself for seedlings height and Avrig for seedlings diameter. Hierarchical cluster illustrated an appropriate linkage between the −30 °C and −40 °C treatments, and the Principal Component Analysis showed an appropriate linkage between provenances Valea Bistrei and Sohodol.

Discussion

Forest ecosystems are frequently exposed to various abiotic stress factors, which negatively affect their growth, resistance, or even survival (Niinemets, 2010; Teshome *et al.*, 2020), and silver fir is often subjected to abiotic stressors (Todea *et al.*, 2020; Morar *et al.*, 2023). In forestry, silver fir is frequently studied with respect to its significant decline that has been periodically noticed in most of the native fir distribution range (Konôpková *et al.*, 2018). Plant photosynthetic processes are highly sensitive to environmental changes and depend on multiple aspects (Stirbet and Govindje, 2011). Low-temperature stress increases reactive oxygen species (ROS) in plant metabolic pathways, reduces tolerance to antioxidant enzymes, damages protein and DNA, accelerates chlorophyll decomposition in leaves, and overall slows down plant growth (Varela *et al.*, 2016; Liu *et al.*, 2019b).

In the current study it was noticed that the cold stress exposure at various low temperatures revealed no differences between seedlings from different geographic origins at −20 °C, a finding explained by temperatures in the Romanian fir-growing regions. For seedlings length, Gârda Seacă provenance recorded the highest value for all treatments. This fact can be explained by the fact that Gârda Seacă is a provenance in Alba country, where negative temperatures are often encountered in winter, reaching up to −20 °C or −30 °C.

As for the number of needles, it was noticed that they almost completely felt within the seedlings exposed to −40 °C, showing that the fir seedlings suffered a severe stress at this negative temperature. Thus, this can be considered a reaction of the *A. alba* species under stress conditions, at least for one-year-old seedlings. Our results clearly showed that the low temperature significantly affected the seedlings growth, whereas it was previous noticed a reduction in the growth and development of the fir seedlings (Zhang *et al.*, 2020). Thus, it can be concluded that stress induced by thermal stress determines important physiological reactions in *Abies alba* seedlings (Miron *et al.*, 2018; Csilléry *et al.*, 2020, 2022).

Seedlings exposed to −40 °C were compromised within all provenances investigated. Different other studies clearly showed that low temperature caused decreases in plants growth, significantly slowed the growth, reduced the photosynthesis, altered the internal leaf structure, and destroyed the chloroplast structure, thus negatively affecting the normal growth and development of different plants (Roschanski *et al.*, 2016; Camarero and Gutiérrez, 2017; Zhang *et al.*, 2020). The obvious negative and distractive effect has been demonstrated hereby at a temperature that can be registered in different parts of Europe, even if only as an accidentally occurrence.

For the conifers, being considered species with a slow growth, long-term treatments are generally required to detect the effects of the applied stress (Șchiop *et al.*, 2015). Although the silver fir is considered a slow-growing species, especially in the first years of life (Wolf, 2003), in our experimental conditions, one-month treatments over one-year-old seedlings were sufficient to detect significant relative reductions in some growth parameters, such as stem length, stem diameter, root weight, or the number of needles, compared to the unstressed plants. A significant reduction of the number of needles in response to temperature drop was also noticed for all populations. Therefore, in our experimental conditions, silver fir does not seem to be resistant to −40 °C at the seedling stage.

The electroconductivity of soil under thermal (cold) stress suggested the soil's capacity to conduct electrical currents when exposed to low temperatures. Cold stress can influence the soil's electrical conductivity, impacting its physical and chemical characteristics. Temperature has a significant impact on salt solubility. Solubility increases with temperature; conversely, solubility decreases with temperature. The EC is lower when precipitation of salt causes a decrease in solubility.

In our experimental conditions, it is evident that silver fir exhibits limited resistance to thermal stress the seedling stage, aligning with previously reported observations in adult trees. This is evidenced by the clear inhibition of seedling growth under stress treatments. Notably, it is important to highlight the substantial variation in growth inhibition patterns among the studied populations. This observation aligns with prior research indicating pronounced differentiation in silver fir populations concerning growth, ecophysiological, and biochemical traits. Such distinctions are attributed to disjunctions in its distribution, as indicated by the effects of the applied stress, as demonstrated in earlier studies with other stress factors (Șchiop *et al.*, 2015; Todea *et al.*, 2019).

Assessment of the diversity of seedlings traits variation is of great relevance to the conservation of genetic resources and management of silver fir (*A. alba*) populations (Todea Morar *et al.*, 2020; Puglielli *et al.*, 2022). Such a results may have theoretical and practical importance for the future silver fir breeding programs in Romania and also for the afforestation programs, especially in the context of current climate changes. Therefore, we conclude that the hereditary basis (provenance) is very important in the reaction of this species to thermal stress conditions, so we can affirm that silver fir presents a relatively high plasticity to thermal stress depending on the origin.

Conclusions

The study highlighted correlations between the effects of cold on the growth of silver fir seedlings, and highlighted some valuable sources for future strategies of species breeding and capitalize of forest genetic resources. Climate change mitigation regarding forest ecosystems is a key topic in forestry. Thus, observations of physiological processes are necessary because they enable modelling and prediction of tree responses to rapid climate change and therefore make possible to identify populations with good growth and resistance/adaptability to various stress factors. These evaluations could enhance the silvicultural strategy of the species and help to identify potential valuable seedlings sources.

Authors' Contributions

Conceptualization, I.M.M., A.M.T. and A.F.S.; methodology, I.M.M., M.B. and F.I.; software, A.F.S. and F.I.; validation, M.B. and F.I.; formal analysis, I.M.M., C.D., and A.M.T.; investigation, I.M.M., C.D. and A.M.T.; resources, I.M.M.; data curation, I.M.M and C.D. writing—original draft preparation, I.M.M. and C.D.; writing—review and editing, M.B. and A.F.S.; visualization, C.D. and F.I.; supervision, M.B. and A.F.S.; project administration, I.M.M.; funding acquisition, I.M.M. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interests

The authors state that there are no conflicts of interest associated with this article.

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