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Physiological Indices of Five Hybrid Larch Seedlings Under Low-Temperature Stress

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Abstract: Larch is a cold-temperate tree species native to the northern hemisphere and tolerant to low temperatures. It is one of the most significant timber species in Northeast China. This study examined growth changes in hybrid larch seedlings from five lines to explore the physiological responses of these seedlings to low-temperature stress. Using 8-month-old hybrids of larch seedlings, we subjected the plants to cold stress at 4 °C and freezing stress at −20 °C over three periods of 6, 12, and 24 h, and treatment at 25 °C was used as a control. Results showed that significant correlations were found among the growth indicators, with larch line 1306 having the lowest incremental growth indicators, the largest root-to-crown ratio, and better cold tolerance than the other larch lines. The levels of soluble sugars (SSs), soluble proteins (SPs), malondialdehyde (MDA), and relative electrolyte leakage (REC) increased significantly in all lines under low-temperature stress. The activities of superoxide dismutase (SOD) and catalase (CAT) showed variation over time. Significant correlations were found between MDA and REL, SS, SR, Pro, CAT, and SOD in most of the lines; no significant correlation was found between MDA and the other indices in lines 1301 and 1309; and significant correlations were found between most of the physiological indices in line 1306.

Keywords: hybrid larch; low-temperature stress; physiological index

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1. Introduction

Larix olgensis is a fast-growing coniferous timber species that is widely cultivated in Northeast China [1]. The larch tree has a well-developed root system and tall branches. Larch plantations play an important role in timber production and provide ecological benefits in China [2]. In recent years, climate change has led to variable spring weather and more frequent extreme events, significantly impacting the expected growth and development of plants [3]. For instance, in the spring of 2010, extended periods of low temperatures in Heilongjiang Province led to the death of a considerable number of plants, the majority of which were classified as level IV or above [4]. Therefore, it is important to study the physiological response of larch to low-temperature stress and propose more efficient cultivation and maintenance methods for this species.

Temperature is an important environmental factor influencing the geographical distribution and growth development of plants. Low-temperature stress includes both chilling injury (below 10 °C) and freezing injury (below 0 °C), and it represents one of the most significant abiotic stresses that plants face. Low-temperature stress induces changes in cell membrane lipid composition, affecting membrane fluidity [5]. After experiencing low-temperature stress, plants produce a large amount of superoxide radicals (O^{2-}), which triggers stress-resistance responses such as the activation of superoxide dismutase (SOD) and catalase (CAT), thereby alleviating the damage caused by low temperatures [6]. In addition, the content of soluble proteins (SP), soluble sugars (SS), and proline (Pro) tends to increase under low-temperature stress [7].

It has been demonstrated that physiological indicators correlate directly or indirectly with the cold tolerance of plants. When exposed to low-temperature stress, different species exhibit different levels of cold tolerance, and different genotypes of the same species even show distinct responses to specific low-temperature conditions [8].

At present, there is extensive research on the cold resistance of plants; however, there exists a relatively limited number of studies specifically focused on the cold resistance of larch. Therefore, five hybrid larch lines were used as study materials to record the trend of growth changes during the seedling period and to detect the changes in various physiological indices by simulating low-temperature stress. We aimed to explore the response of some physiological indices of different hybrid larch lines to low temperatures, compare the differences in cold tolerance among the progeny of different crosses in combination with the growth changes during the cultivation period, and to carry out a preliminary study on the cold tolerance of five hybrid larch lines. This study is of significant importance for evaluating the cold resistance of larch seedlings and provides a basis for the selection and breeding of larch lines.

2. Materials and Methods

2.1. Plant Materials

Outstanding individual plants selected from the seed orchard at Qingshan Forest Farm in Heilongjiang Province (East Longitude 133°53′28″–133°58′05″, North Latitude 46°38′56″–46°44′20″) were cross-pollinated to obtain hybrids, which were then sown to produce hybrid seedlings. The hybrid lines were numbered as 1301, 1305, 1306, 1307, and 1309, with the maternal parent lines and paternal parent lines outlined in Table 1. The seeds were sown in the laboratory of Northeast Forestry University on 14 May 2023. The hybrid larch seedlings were subsequently cultivated in a plant light culture room at 25 ± 1 °C, with a daily light duration of 16 h and a light intensity ranging from 40 to 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$. During this period, the seedlings were regularly watered. Following germination, the seedlings were transplanted and grown for an additional 60 days and subsequently moved to a laboratory greenhouse. The seedlings were cold-stressed 180 days after transplanting.

Table 1. The five hybrid larch lines have both maternal and male plant parents.

Line	Female Parents	Male Parents
1301	*Larix olgensis* 73-2	*Larix olgensis* 73-3
1305	*Larix olgensis* 73-3	*Larix olgensis* 73-14
1306	*Larix olgensis* 73-14	*Larix olgensis* 73-1
1307	*Larix olgensis* 73-14	*Larix olgensis* 73-3
1309	*Larix olgensis* 73-14	*Larix olgensis* 73-34

2.2. Low-Temperature Treatment

Uniformly growing seedlings were selected for low-temperature stress. Uniformly growing seedlings were selected for low-temperature stress treatment. In simulated low-temperature environments of 4 °C and −20 °C, the seedlings were subjected to stress for 6 h, 12 h, and 24 h, using room temperature conditions as the control treatment. Each lineage comprised 7 low-temperature stress treatments, with 3 seedlings for each treatment. Following stress treatment, we obtained a sample of needles, froze them with liquid nitrogen, wrapped them in aluminum foil, labeled them, and placed them in a −80 °C ultra-low-temperature freezer for use in future experiments.

2.3. Biomass and Growth Index Determination

We rinsed the soil from the roots of the larch seedlings thoroughly with sterile water, recorded the fresh weight of the entire plant, and then cut them into two sections at the base diameter. The weight of the aboveground and belowground parts was recorded separately.

We placed the aboveground and underground parts in paper bags and dried them in an oven at 110 °C for 30 min to fix them and then continued to dry them at 85 °C until a constant weight was reached. The dry weight of the above- and belowground parts of the plant was determined and used to calculate the total biomass of the whole plant and the root-to-crown ratio.

After the seedlings were transplanted, the height of the hybrid larch seedlings was measured from the base to the highest point of the plant using a meter ruler as the initial plant height. The height increment was calculated as the measured height minus the initial height. After the seedlings were transplanted, the diameter of the rootstock at 1 cm from the base of the main root of the seedling was measured using a vernier caliper as the initial ground diameter. Diameter increments were calculated by subtracting the initial diameter from the measured diameter. Thereafter, the growth parameters of the different lines were measured at regular intervals of 30 days.

2.4. Measurement of Physiological Parameters

Relative conductivity (REC) was measured by taking 0.5 g of seedling branches following stress, using the method of He Liming [9]. Soluble sugar (SS) content, soluble protein (SP) content, proline (Pro) content, and malondialdehyde (MDA) content were determined using the anthrone colorimetric method, Coomassie Brilliant Blue G-250 method, ninhydrin colorimetric method, and thiobarbituric acid method, respectively [10]. Superoxide dismutase (SOD) activity was measured using the nitro blue tetrazolium chloride method, and catalase (CAT) activity was assessed using the ammonium molybdate method [11]. Correlation analyses of the physiological indices of the five hybrid larch species were carried out using IBM SPSS 26.0.

2.5. Statistical Analysis

We conducted a variance analysis (ANOVA) using IBM SPSS 26.0, followed by Duncan's multiple range test. All experimental data are presented as the mean ± standard error (SE), with the significance level set at $p \leq 0.05$. Tables were generated using Microsoft Excel 2010 and Microsoft Word 2010, and images were plotted using Origin 2021.

3. Results

3.1. Variation in Growth Indicators and Biomass

Significant differences ($p < 0.05$) were observed in the mean total increments of height, ground diameter, and crown spread of larch seedlings belonging to different lines of hybrid larch, as illustrated in Table 2. The highest increments in seedling height and ground diameter were recorded for line 1301, followed by line 1309. Line 1306 exhibited the lowest increases in both height and ground diameter. Line 1301 achieved a plant height of 20.61 cm, which was significantly higher than the increments observed in the other four lines. The lowest recorded plant height and ground diameter for line 1306 were 9.69 cm and 1.22 mm, respectively, which were 52.98% and 35.45% lower than those observed for line 1301. The greatest increase in crown width was observed in line 1309, at 6.28 cm, which was 88.02% higher than that of line 1305, which exhibited the lowest increase in crown width. As illustrated in Table 3, the aboveground, belowground, and total dry weight of hybrid larch seedlings from all lines were highest in line 1301, which exhibited a biomass significantly higher than that of the lowest biomass line, 1307, by 330.51%, 335.71% ($p < 0.05$), and 331.51%, respectively. The root-to-crown ratio of dry weight was significantly higher in line 1306 compared to the remaining lines, with increases ranging from 108.51% to 171.75% ($p < 0.05$).

Table 2. The growth index increments of the different lines of hybrid larch seedlings.

Line	Plant Height (cm)	Root Collar (mm)	Crown Width (cm)
1301	20.61 ± 2.2421a	1.89 ± 0.3716a	5.81 ± 0.4886a
1305	13.06 ± 1.0937c	1.71 ± 0.3074ab	3.34 ± 0.9153c
1306	9.69 ± 1.4494d	1.22 ± 0.2804c	4.44 ± 0.8394b
1307	13.80 ± 0.9578c	1.51 ± 0.2563b	4.54 ± 1.2220b
1309	16.79 ± 1.6704b	1.73 ± 0.5157ab	6.28 ± 1.1599a

Note: Different lower-case letters in the same indicator for the five line indicate significant differences between line ($p < 0.05$).

Table 3. Increment of biomass in seedlings of different hybrid larch lines.

Line	Shoot Dry Weight (g)	Dry Root Weight (g)	Plant Dry Weight (g)	Root-to-Shoot Ratio
1301	2.54 ± 0.1661a	0.61 ± 0.0312a	3.15 ± 0.1953a	23.85 ± 0.6131b
1305	1.13 ± 0.0603b	0.21 ± 0.0289c	1.33 ± 0.0874b	18.30 ± 1.6686c
1306	0.73 ± 0.0600cd	0.36 ± 0.0351b	1.09 ± 0.0950c	49.73 ± 0.7792a
1307	0.59 ± 0.0557d	0.14 ± 0.0200d	0.73 ± 0.0755d	23.66 ± 1.2666b
1309	0.83 ± 0.0686c	0.16 ± 0.0172d	0.99 ± 0.0859c	18.79 ± 0.5282c

Note: Different lower-case letters in the same indicator for the five line indicate significant differences between line ($p < 0.05$).

3.2. Effects of Osmoregulatory Substances in Larch Under Low-Temperature Stress

In the simulated low-temperature stress of 4 °C cold damage and −20 °C freezing damage, the effects on SP, SS, and Pro in the five hybrid larch lines under different time stresses are shown in Figures 1–3. The SP content in the majority of the hybrid larch lines showed an irregular trend, with more pronounced fluctuations occurring between 6 and 12 h. Both SS and Pro showed an increasing-decreasing single-peak trend. The changes in SS and Pro under −20 °C low-temperature stress are more pronounced than those under 4 °C low-temperature stress, with significant fluctuations observed during the 0 to 12 h period. When subjected to 4 °C low-temperature stress, the Pro content of line 1305 varies between 50.77 $\mu g \cdot g^{-1}$ and 61.76 $\mu g \cdot g^{-1}$. Under −20 °C low-temperature stress, the variation range is from 55.36 $\mu g \cdot g^{-1}$ to 147.37 $\mu g \cdot g^{-1}$. The maximum Pro content at −20 °C low-temperature stress is 2.38 times that of the highest Pro content at 4 °C low-temperature stress.

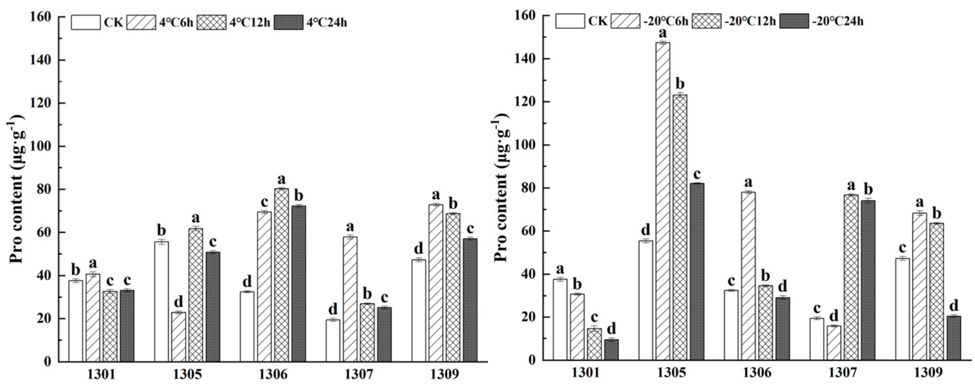

Figure 1. Effects of low-temperature stress on Pro content in larch seedlings. Different lower-case letters in the same family indicate significant differences between treatments at different times ($p < 0.05$).

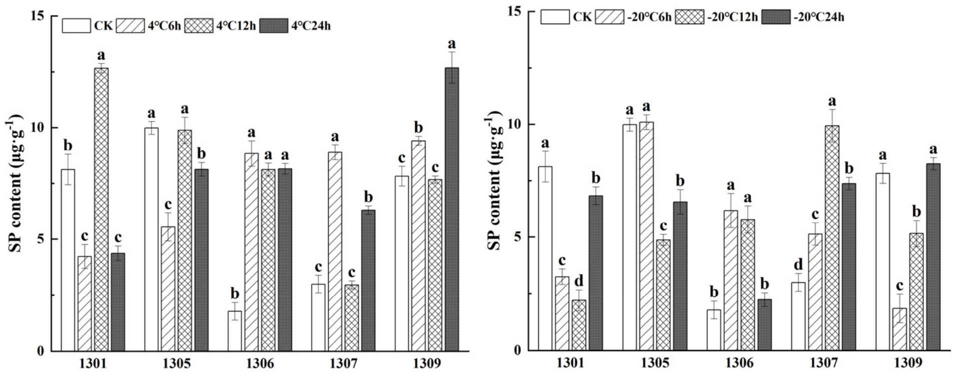

Figure 2. Effect of different temperatures and time of stress on SP of larch seedlings. Different lower-case letters indicate significant differences ($p < 0.05$).

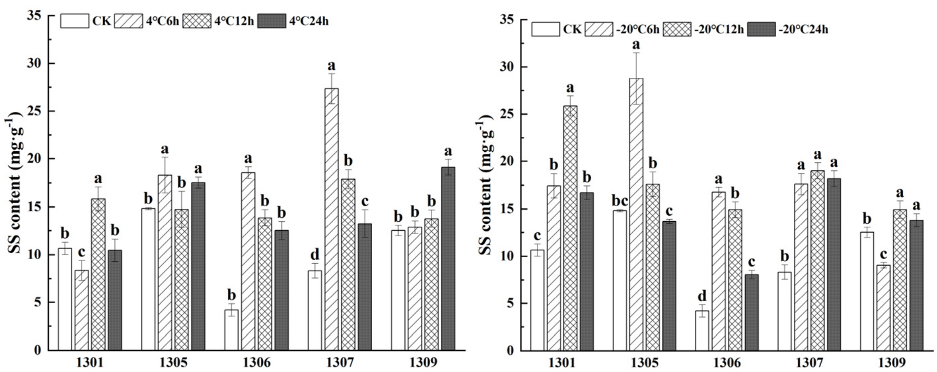

Figure 3. Effect of different temperatures and time of stress on SS of larch seedlings. Different lower-case letters in the same family indicate significant differences between treatments at different times ($p < 0.05$).

3.3. Effect of Larch Protective Enzyme Activities Under Low-Temperature Stress

Figures 4 and 5 illustrate the impact of five hybrid larch lines on the activities of superoxide dismutase (SOD) and catalase (CAT) under low-temperature stress at 4 °C and −20 °C, over different time intervals. The CAT activity in lines 1306 and 1307 was significantly higher than that of the control (CK) by 4.96 times and 2.76 times after 6 h of stress at 4 °C; in comparison, line 1309 showed a 3.31 times increase after 12 h of stress at 4 °C. In line 1307, the variation in SOD activity was relatively small for the first 12 h of low-temperature stress at both 4 °C and −20 °C. However, when the stress duration was 24 h, SOD activity was significantly higher than the control by more than three times.

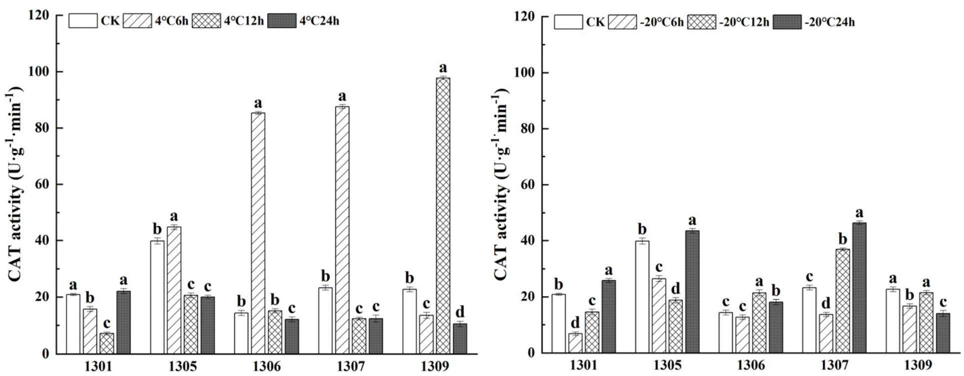

Figure 4. Effect of different temperatures and time of stress on CAT of larch seedlings. Different lower-case letters in the same family indicate significant differences between treatments at different times ($p < 0.05$).

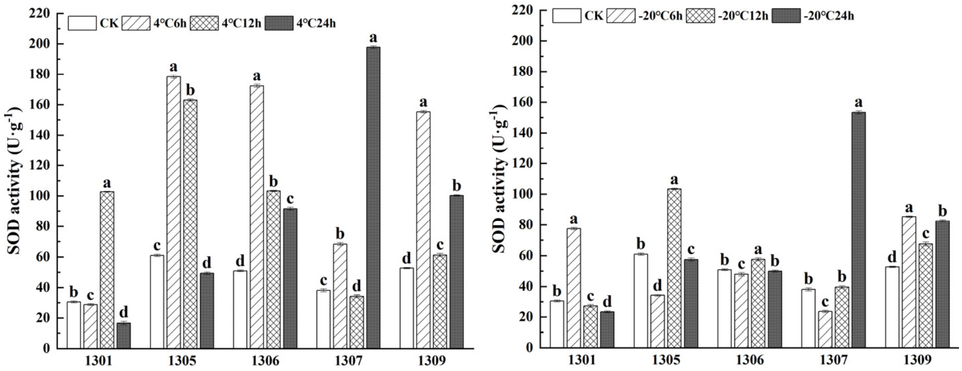

Figure 5. Effect of different temperatures and time of stress on SOD of larch seedlings. Different lower-case letters in the same family indicate significant differences between treatments at different times ($p < 0.05$).

3.4. Effects of Membrane Peroxidation in Larch Under Low Temperature Stress

As shown in Figures 6 and 7, the REC and MDA content of the five hybrid larch lines showed a gradually increasing trend with the prolonged stress duration. After treatment for 24 h at 4 °C and −20 °C, the relative conductivity compared to the CK changed within the ranges of 3.47%–13.36% and 41.16%–51.15%, respectively. The five lines of hybrid larch under 24 h of stress had a relative conductivity that was significantly higher than that of the other treatment groups. Additionally, the REC value of line 1309 was significantly higher than that of the other lines. The lowest REC values were recorded in line 1301 (27.95% at 4 °C and 47.00% at −20 °C); in comparison, the highest average REC values were recorded in line 1309 (39.33% at 4 °C and 66.49% at −20 °C). Lines 1301, 1305, 1307, and 1309 reached the highest MDA content at 24 h of stress, which was significantly higher than those of the CK and other stress treatments. In contrast, line 1306 demonstrated a declining trajectory following its peak at 6–12 h; however, its MDA content remained considerably elevated compared to that observed in the CK.

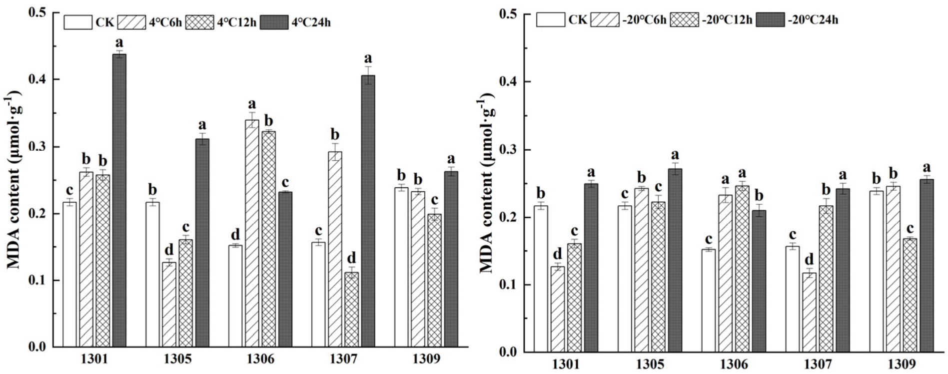

Figure 6. Effect of different temperatures and time of stress on MDA of larch seedlings. Different lower-case letters in the same family indicate significant differences between treatments at different times ($p < 0.05$).

3.5. Correlation Analysis of Physiological Indicators

As illustrated in Figure 8, the physiological indicators measured for the five hybrid larch lines were standardized to obtain a correlation coefficient matrix, which showed significant differences among the different hybrid progeny lines. In line 1301, REL and Pro showed a highly significant negative correlation ($p < 0.01$), while CAT and SOD also exhibit a significant negative correlation ($p < 0.05$). In line 1305, MDA and SOD were extremely significantly negatively correlated ($p < 0.01$). In line 1306, MDA showed a significant positive correlation ($p < 0.05$) with SS, SR, and SOD, and SR showed a significant positive correlation with SS and Pro, and CAT showed a significant positive correlation with SOD.

In line 1307, CAT was positively correlated ($p < 0.05$) with SS and MDA, and in line 1309, SS and SR exhibited a significant positive correlation ($p < 0.05$).

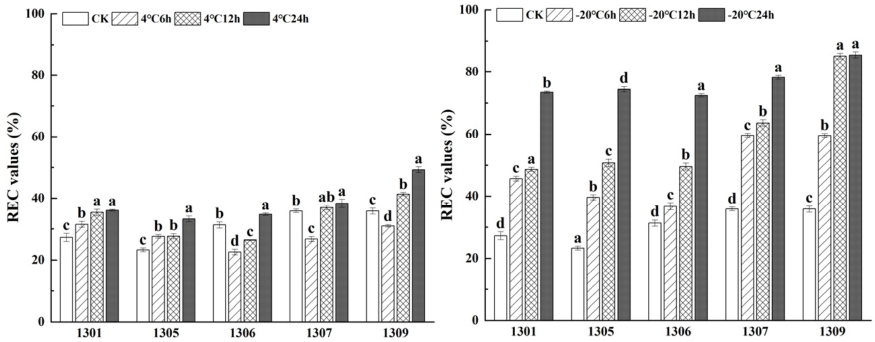

Figure 7. Effect of different temperatures and time of stress on MDA of larch seedlings. Different lower-case letters in the same family indicate significant differences between treatments at different times ($p < 0.05$).

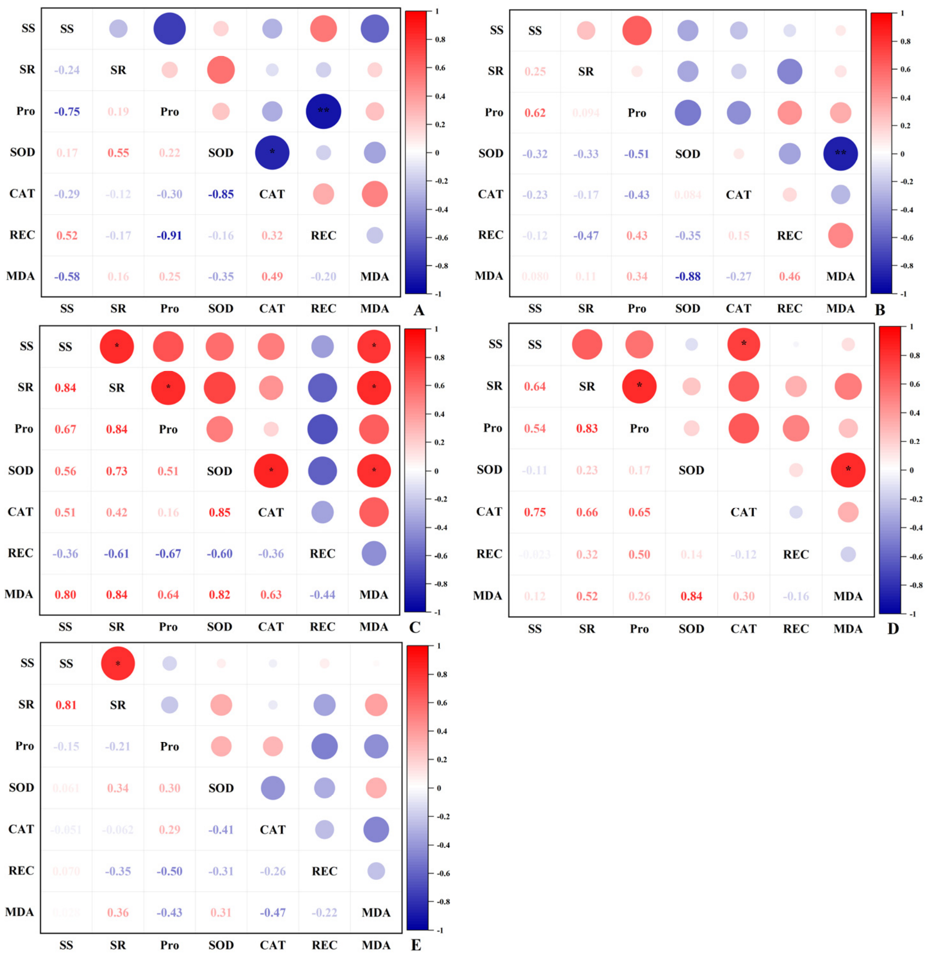

Figure 8. Correlation between different lines and physiological indicators. * $p < 0.05$ = significantly correlated; ** $p < 0.01$ = extremely significantly correlated. Subfigures (**A**–**E**) are lines 1301, 1305, 1306, 1307, and 1309, respectively.

4. Discussion

The growth and morphological indicators of plants can be used to intuitively assess their growth status and the extent to which seedlings are able to acclimate to their environment [12]. The most intuitive and important indicators reflecting the growth status, characteristics, and robustness of seedlings are crown width, plant height, and root collar diameter [13]. The plant height and root collar diameter of line 1301, in addition to the crown diameter of line 1309, were found to be significantly higher than those of the other lines. Conversely, the plant height and ground diameter of line 1306 were observed to be significantly lower than the growth increments of the other lines. Biomass is also responsive to the accumulation of assimilation products and the growth and development process of seedlings [14]. The highest biomass was noted in the aboveground, belowground, and whole plant dry weights of line 1306, which was similar to that of line 1309. In contrast, the lowest biomass was observed in the aboveground, belowground, and whole plant dry weights of line 1307. However, the root-to-crown ratio of line 1306 was the largest among the lines, indicating that this line has a well-developed root system and is better able to absorb soil nutrients. Xie Hefeng studied the growth and cold resistance of *Populus delotides* Marsh asexual lines by showing that their biomass was negatively correlated with cold resistance [15]. In this study, hybrid larch from five lines showed that line 1306 had relatively shorter height and thinner stems but better resistance to stress, whereas line 1301 exhibited poorer stress resistance.

Soluble proteins (SPs) have the capacity to regulate cellular osmotic potential and protect the stability of biofilms. Furthermore, they can lower the cytoplasmic freezing point by enhancing cellular water retention capacity. In conjunction with SSs, which act as important osmotic regulators under low-temperature stress, these factors are indicative of plant cold resistance [16]. Compared to the CK, low-temperature stress significantly increased the mass fraction of SSs and SPs in the hybrid larch seedlings, aligning with the findings of previous studies [17]. The accumulation of substantial quantities of Pro helps to maintain the intracellular environment in a relatively stable state. Consequently, the Pro content in plants can serve as a measure of the plant's stress tolerance to a certain extent [18]. In this experiment, the Pro content of the five lines of hybrid larch seedlings exhibited a trend of initial increase followed by a decline as the duration of low-temperature stress was prolonged. This finding is consistent with the results reported by Zhang [19] and Soloklui [20].

Both SOD and CAT play a crucial role as reactive oxygen scavengers when plants are under stress [21]. Biao Li, in a study on *Ziziphus jujuba* var, found that the activities of POD and CAT initially increased and then decreased after exposure to low-temperature stress [22]. Leaves of *Dimocarpus longan* seedlings showed a similar trend, with POD, SOD, and CAT activities initially increasing and then decreasing as low-temperature stress was prolonged [16]. In this experiment, the SOD activity and CAT activity of five strains of hybrid larch seedlings showed a unimodal trend of increase first and then decrease under different time and temperature stresses, consistent with previous studies. The physiological indices of each line behaved differently under varying stress conditions, indicating that plants face multiple factors in resisting low temperatures, and there is not simply a synergistic effect among these factors [23].

In the event of adverse conditions affecting plants, damage occurs to the cell membranes, which results in subsequent leakage of electrolytes from the cells. We found that as the degree of cell membrane damage increased, the relative conductivity also rose. MDA is a product of membrane lipid peroxidation and can be used to reflect the extent of lipid peroxidation in the cell membrane and the intensity of a plant's reaction to stressful environmental conditions. The findings of this study indicate that the relative conductivity and MDA content of larch seedlings from different lines exhibited a gradual increase with the decrease in stress temperature and the prolongation of stress time. These findings suggest that as the stress temperature decreases and the duration of stress increases in hybrid larch seedlings, the damage to the cell membranes of the stem sections, in addition to the subsequent change in the permeability of intracellular electrolytes, leads to an elevation in relative conductivity. Concurrently, low-temperature stress resulted in elevated peroxidation of the cell membranes of the hybrid larch seedlings, accompanied by a corresponding increase in the content of MDA. Sun [24] and Wang [25] demonstrated that relative conductivity and MDA content exhibit an upward trajectory with the prolongation of stress time under differing low-temperature treatment conditions, a finding that aligns with the outcomes of the present study.

The mechanisms of various physiological indicators in plants in response to low-temperature stress are complex and cannot be directly evaluated by a single indicator to assess their cold resistance. As used in this study, correlation analysis can reveal the degree of correlation between different indicators, thus providing a basis for understanding the changes in various indicators of hybrid larch seedlings in response to low-temperature stress. Our correlation analysis results indicate that under low-temperature stress, most indicators among the five hybrid larch lines show a correlation. The MDA content of most lines is significantly positively correlated with SS, SP, SOD, and CAT; in comparison, SS is significantly positively correlated with SR, Pro, and CAT. These findings indicate that changes in MDA content can, to some extent, predict the increasing or decreasing trends of SS content, SP content, SOD activity, and CAT activity, and the stronger the correlation among them, the more pronounced this trend becomes.

5. Conclusions

Once transplanted, the five hybrid larch line seedlings displayed different distributions of plant height, root collar diameter, crown width, and biomass under the same environmental conditions. Line 1301 exhibited the largest increments in growth indices and biomass, whereas line 1306 had the smallest increments in these measures. However, line 1306 had the highest root-to-shoot ratio, which indicates better cold tolerance. The SS content and SP content in hybrid larch seedlings of all lines were significantly increased by stress treatments at different temperatures and for different times, whereas there were no significant trends in SOD and CAT activities. Additionally, MDA content and REC values increased significantly. There was no significant correlation between MDA content and the indicators in lines 1301 and 1309, which exhibited a weaker ability to cope with adversity and lower resistance compared to the other lines. Line 1306 is more cold resistant and showed significant correlations among multiple physiological indicators under low-temperature stress, indicating its stronger ability to cope with such stress. By analyzing the growth indicators and changes in physiological indicators after low-temperature stress in the different hybrid larch lines, we can effectively assess the cold resistance of seedlings from different lines in low-temperature environments. Analyzing the differences among different lines identify traits that can be used for genetic improvement. By combining the growth and physiological indicators of larch seedlings, we can select lines with strong cold resistance and further breed larch varieties that are more suited to cold environments.

Author Contributions: S.L. conceived and designed the experiments; H.Z. provided the funding; Y.N. performed the experiments, analyzed the data, and prepared the figures and tables; W.Z. reviewed drafts of the paper; C.C., X.Z. (Xinxin Zhang), X.Z. (Xin Zhao), Y.L. and C.W. completed the formal analysis and investigation. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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