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Frost hardiness of Finnish plus tree progenies of Scots pine from seed orchards in Finland and Ukraine

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Abstract

To avoid the surrounding pollination, seed orchards of Finnish plus trees have been established in Ukraine, i.e., far from their natural distribution. However, it is unknown whether the pollination site affects the frost hardiness (FH) of the progenies and thus their use in forest regeneration in Finland. Using whole-plant freezing tests, we examined the FH of plus tree seed orchard progenies in Finland and Ukraine, with three lots of seeds from each orchard, twice during cold acclimation in controlled conditions. The FH of needles, as assessed immediately after the freezing tests, was higher in the Finnish than Ukrainian seed orchard progenies. The variation in the FH of needles was high among the progenies, ranging from -44 to -79 °C by relative electrolyte leakage (REL), and from -29 to -46 °C by chlorophyll fluorescence (CF). However, when FH was assessed based on the growth of shoots and roots in the greenhouse after three weeks, the differences between the progenies and orchards disappeared. The FH was then between -10 and -15 °C based on shoot growth, between -6 and -12 °C based on root growth, and between -8 and -16 °C based on the number of new root tips. The results imply an epigenetic effect on FH of needles but no effect of progeny or orchard on FH of roots. In summary, it seems to be possible to use seed produced in southern Ukraine in practical reforestation actions in southern Finland.

Keywords Chlorophyll fluorescence · Cold acclimation · Progeny · Relative electrolyte leakage · Root morphology · Visual damage scoring

Introduction

Scots pine (*Pinus sylvestris* L.) has the widest geographic distribution of all pine species, extending from Spain in the south to northern Scandinavia in the north, and from Scotland in the west to the eastern part of Russia in the east (Mirov 1967). In Finland alone, the length of the growing season, in terms of temperature sum of days with an average temperature above 5 °C, in the Finnish distribution area of Scots pine varies from about 170 days at latitude 60° N to less than 120 days at latitude 69° N (Hurme et al. 1997; Briceño-Elizondo et al. 2006). Growth in diverse environmental conditions is possible through plastic responses, genetic differentiation, or epigenetic inheritance (Johnsen et al. 1996; Skråppa et al. 2007). Indications of local genetic adaptation are obtained from seed transfer experiments (Eriksson et al. 1980; Persson 1994). When southern provenances were transferred to the north, their survival was reduced (Beuker 1994). On the other hand, northern provenances transferred to the south had increased survival, but their growth was less

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than the growth of the local southern provenances (Persson and Ståhl 1990).

In forest management and silviculture, the aim is to increase forest productivity by breeding plus trees of Scots pine that have proven high production and good wood quality in their natural stands (Haapanen et al. 2016; Egbäck et al. 2018). At northern latitudes, the production of trees is limited by short growing seasons and long cold winters (Berlin et al. 2016). For a high survival rate and yield, trees must therefore compromise between the efficient use of the short growing season and the right timing of the initiation of frost hardening and de-hardening in the autumn and spring, respectively. Early growth initiation and/or late growth cessation predispose trees to frost damage. The genes of plus trees are thought to include a coded capability of acclimation and adaptation to high seasonal variation in environmental conditions, especially temperature, photoperiod, and the quality and quantity of light (Andersson Gull et al. 2018; Alakärppä et al. 2019). They are therefore also thought to fit breeding purposes well.

Trees time their growth and reproduction to coincide with favorable conditions, depending on the genotype (Weiser 1970; Christersson 1978; Bonan and Shugart 1989). In the juvenile phase, first-year seedlings of Scots pine have a free growth pattern, and their bud set and cold hardening are then determined by the joint effect of longer nights and a decreasing temperature (Koski and Sievänen 1985; Repo 1992; Hurme et al. 1997; Repo 1992). However, their growth pattern switches to being predetermined by age, whereupon the cessation of shoot elongation takes place when a certain temperature sum has accumulated (350–450° days; 5 °C as the threshold for daily mean temperature), with some variation between provenances (Koski and Sievänen 1985; Repo et al. 2000). In the provenance trials, the frost hardening of the aboveground parts of both first-year seedlings and older trees growing on the same site started earlier in the northern than southern provenances, which indicated a strong genetic basis of these events (Hurme et al. 1997; Beuker et al. 1998; Repo et al. 2000; Nilsson 2001).

To produce high-quality seeds for forest regeneration, seed orchards of grafted (cloned) plus trees of Scots pine have been established in various locations in Finland (Lehtinen and Pulkkinen 2017). However, in these orchards, surrounding pollination may reduce the genetic quality of the seeds. To avoid this effect, Finnish plus tree seed orchards were established in Ukraine, where there is no surrounding pollination, i.e., they are far from the natural native distribution, but are located in more favorable growth conditions more than 1600 km south of Finland. However, there are no comparative studies of whether the FH of seedlings raised using seeds from Finnish and Ukrainian orchards differ.

Among the organs, roots have been considered the most frost-susceptible organ, and in cold areas, they may be

damaged during overwintering if the protective snow cover is missing (Sakai and Larcher 1987; Colombo et al. 1995; Drescher and Thomas 2013; Domisch et al. 2018). In a recent study with short-term freezing tests, some parts of the root system of frost-hardened Scots pine seedlings tolerated lower temperatures than was previously thought (Di et al. 2019). This suggested that root damage might not necessarily be the primary reason for a decline in growth. However, when the roots were exposed to frost for a longer period, fine root mortality was found to increase (Tierney et al. 2003). Although significant differences have been found in the FH of above-ground organs between genotypes (Beuker 1994; Repo et al. 2000; Nilsson 2001), there are no previous studies of whether there are differences in the FH of roots.

The aim of this study was to compare the FH of the different organs and progenies of Scots pine raised using seeds from grafted plus tree seed orchards in Finland and Ukraine (three in each), the progenies of both orchards having the same mother trees. The assessment took place using whole-plant freezing tests twice at one-month intervals during cold acclimation in controlled conditions. We hypothesized that: (i) the frost hardiness (FH) of above-ground parts of Scots pine should be similar in Finnish progenies and in Ukrainian progenies within the same progeny; and (ii) there were no differences in the FH of roots between the seed orchard progenies.

Materials and methods

Plant material and experimental design

The study was conducted using 1,620 first-year seedlings of Scots pine (*Pinus sylvestris* L.) raised from six lots of seeds in the research nursery at the Haapastensyrjä unit of Natural Resources Institute Finland (Luke). Three lots of open-pollinated seeds were collected from seed orchards in both Viiala in Finland and in Vinnytsia in Ukraine (Table 1). As a result, there were two geographic origins with three progenies each, with 270 seedlings in each progeny. The progenies were labelled with the letters F or U, corresponding to Finland or Ukraine, respectively, and a number from 1 to 3, referring to the mother tree that was the same for the Finnish and Ukrainian progeny, and labelled by the same number accordingly (Table 1).

The seeds were sown in pre-fertilized peat (Kekkilä Oy, Tuusula, Finland) in trays (size 40×60 cm), with 96 seedlings in each container on 16 February 2016, at the Haapastensyrjä nursery. After germination, the seedlings were transplanted in PL-81F containers (cell volume 85 cm³, 9×9=81 cells per container, 546 m⁻², Lännen Oyj, Iso-Vimma, Finland) on 26 April 2016. The seedlings were raised in a climate-controlled growth chamber with a photoperiod

Table 1 The location of plus tree seed orchards and the site of pollination in Ukraine (U) and Finland (F), and the origin of mother trees in the seed orchards. The labels of the progenies from the seed orchards in Ukraine and Finland are indicated

Location of seedling nursery	Location of seed orchard	Location of the mother trees	Label of progeny
Haapastensyrjä (Luke), Finland (60° 36' N, 24° 25' E 54 m asl.)	Vinnytsia, Ukraine (U) (49°23' N, 28° 07' E, 283–288 m asl.)	Suomenniemi, Finland (61° 25' N, 27° 05' E, 90 m asl.)	U1
		Sulkava, Finland (61°45' N, 28° 28' E, 90 m asl.)	U2
		Heinola, Finland (61°20' N, 25°52' E, 100 m asl.)	U3
	Viiala, Finland (F) (61°21' N, 23°65' E, 85 m asl.)	Suomenniemi, Finland (61° 25' N, 27° 05' E, 90 m asl.)	F1
		Sulkava, Finland (61° 45' N, 28° 28' E, 90 m asl.)	F2
		Heinola, Finland (61° 20' N, 25° 52' E, 100 m asl.)	F3

of 18/6 h (day/night) with PAR 325 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Philips Master Agro 400W), an air temperature of 20 °C/15 °C (day/night), and a relative humidity (RH) of 80%/90% (day/night) for 14 weeks before the start of the cold acclimation programme.

The first phase of cold acclimation (H1) took place in the same growth chamber as the raising of the seedlings, and it lasted for four weeks. The H1 phase started by decreasing the air temperature from 20 °C/15 °C (day/night) to 5 °C/5 °C (day/night) in four steps, by 3.8 °C per week in the daytime and 2.5 °C per week at night. At the same time, the RH decreased from 80%/90% (day/night) to 70%/80% (day/night), the PAR decreased from 325 to 66 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and the photoperiod decreased from 18 h/6 h (day/night) to 6 h/18 h (day/night). The second phase of cold acclimation (H2) consisted of the maintenance of the seedlings in the final conditions of H1 for six weeks. At the end of H2, the seedlings were transported to the Biosphere laboratory of the University of Eastern Finland and Luke in Joensuu (62° 61' N, 29° 74' E, 80 m a.s.l.) and set in three replicate growth chambers (PGW36, Conviron Ltd., Winnipeg, MB, Canada) with the same illumination, temperature, and RH conditions as during H2.

Freezing test

Frost hardiness was assessed by controlled freezing tests twice at one-month intervals to test whether there was a change in FH from prolonged storage in constant growth chamber conditions. The first test (T1) started on 3 August 2016, immediately after the seedlings were transferred to Joensuu, and the second test (T2) on 3 September. In both tests, there were six freezing temperatures (−3 °C, −8 °C, −16 °C, −30 °C, −48 °C, and −80 °C) with the control temperature (4 °C). At four of the freezing test

temperatures (−3 °C, −8 °C, −16 °C, and −30 °C) and the control temperature, the treatment took place as with the whole-plant freezing tests but for −48 °C and −80 °C, as the detached needles only. After the freezing tests, the FH of the needles was assessed by the electrolyte leakage method (REL) and chlorophyll fluorescence (CF). The seedlings were then moved to the greenhouse for three weeks to assess the damage according to the growth of the shoots and roots.

The freezing exposures took place in programmable chambers (ARC 300/−55/+20, Arcstest, Espoo, Finland) for the temperatures between −3 and −48 °C, and in one N₂-gas-cooled chamber (GCC-30, Carbolite, Chelmsford, UK) for −80 °C. There were 27 seedlings (three replicates and nine seedlings per replicate) from each progeny at each test temperature. The cooling rate was 2 °C h^{−1}, from 5 to −3 °C, which was maintained for five hours to ensure uniform freezing of the soil in the pots. The cooling then continued at a rate of 2 °C h^{−1} to the target temperature, which was maintained for four hours. The warming rate back to 5 °C was 5 °C h^{−1}. After the freezing exposures, the seedlings were thawed at 5 °C for 3–4 days and then moved to room temperature for one day before the sampling of needles and the assessing of their FH by REL and CF. The growth measurements of shoots and morphological analyses of roots were carried out after three weeks of growth in the greenhouse to obtain whole-plant FH (see below). Different organs were assumed to be affected by freezing stress at different degrees. They were therefore analyzed separately. Needle injuries, as assessed by REL and CF, would appear immediately after freezing stress whereas damage in roots would appear with a delay as a decline of new root tip formation, root growth, and shoot growth. The root attributes were considered as measures of root vitality.

Relative electrolyte leakage of needles (REL)

After the freezing tests, 32 needles were sampled from three seedlings of each of the three replicates and of each progeny by freezing temperatures for the REL test (Repo and Lappi 1989; Di et al. 2019). Ten-millimetre-long samples were cut in the middle of the needles and set in test tubes (eight samples per tube), with four tubes for each of the three sampled seedlings. There were 756 tubes in both sampling sessions. Ten millilitres of distilled water were added to the test tubes, which were shaken (200 rpm) at room temperature for 22 h before the first conductivity measurement (L1) (CDM92 conductivity meter with CDC641T electrode, Radiometer, Copenhagen, Denmark). The samples were then heat-killed at 92 °C for 20 min and shaken for another 22 h before the second conductivity measurement (L2). The relative electrolyte leakage (REL) was defined as:

$$REL = \left(\frac{L1}{L2} \right) \times 100. \quad (1)$$

Chlorophyll fluorescence of needles (CF)

After the freezing tests, 15 needles were sampled from the top of each of the three seedlings from each replicate and progeny by freezing temperatures for the measurement of dark-acclimated (20 min) chlorophyll fluorescence (F_v/F_m) (PAM-2500, Walz, Heinz Walz GmbH, Effeltrich, Germany). The needles were attached side by side to adhesive tape. The measurement gained information about the change in the potential efficiency of the quantum yield of photosystem II ($PSII$) by freezing damage (Öquist and Ögren 1985; Baker 2008; Repo et al. 2006).

Shoot regrowth and biomass

After the freezing tests, six seedlings of each replicate and progeny (540 seedlings in total) from both sampling times were moved to the greenhouse at a temperature of 20 °C, with a photoperiod of 18 h/6 h (day/night), a photon flux density of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (HS400W, Philips, Vantaa, Finland), and an air humidity of between 70 and 80%. After three weeks of growth, the length of the new shoots was measured. In addition, the total above-ground dry mass (including the dry mass of new and old shoots) of each seedling was measured. The new and old shoots (including stem and needles) were dried at 40 °C for one week before the dry weight was measured.

Root morphology

In the final harvest at the end of three weeks of growth in the greenhouse, the roots of seedlings from both sampling times T1 and T2 (a total of 1080 seedlings) were harvested for

root morphology analyses. Each root was carefully separated from the soil, the total root length and the number of root tips were assessed by scanning, and subsequently analyzed using the WinRHIZO program (WinRHIZO 3.1.2, Regent Instruments Inc. Quebec, Canada).

Statistical analyses

The frost hardiness (FH) of different organs was estimated as the inflection point (parameter C) of Eq. (2):

$$y = \frac{A}{1 + e^{B(C-x)}} + D, \quad (2)$$

where y refers to REL, CF, shoot length, root length, root tips, and shoot biomass, x to the exposure temperature, parameters A and D define the asymptotes, and B is the slope at the inflection point (Repo and Lappi 1989). The values of the parameters in Eq. (2) were computed by nonlinear regression analysis. The standard error of parameter C was estimated using bootstrapping. The approximate significance of the differences between progenies and seed orchards was determined by applying a normal distribution and the estimated standard error of the C parameter. The effect of sampling times, orchards on shoot and root length, root tips and shoot biomass by freezing temperatures were analyzed by applying linear mixed models, which included a chamber identifier as a random factor. The mixed model was used to quantify the differences in FH, as assessed under various methods, between different sampling times and orchards. Progeny was treated as a fixed factor except in the models testing the differences between the three progenies. The results were computed using the IBM SPSS procedure MIXED (IBM SPSS 25.0, IBM Co., New York, USA). In multiple comparisons, the significance values were adjusted within each set of comparisons applying the Holm-Bonferroni step-down method (Holm 1979; Lappi and Luoranen 2018; Wu et al. 2019). The adjusted significance values were computed using an R script (R version 3.3.2, R Foundation for Statistical Computing, Vienna, Austria).

Results

Frost hardiness of needles by REL and CF between progenies

According to the REL test, the FH of the needles varied between -44 °C and -79 °C, depending on the progeny (Fig. 1A). The FH of F3 was higher than that of any other progeny in the first freezing test (T1) (Fig. 1A). In the second freezing test (T2), progenies F3, F1, and U3 had higher

FH than U1 and U2, while the FH of F2 lay between the two sampling times (Fig. 1A).

The differences between the progenies in FH were smaller when assessed by CF than by REL in both sampling times (Fig. 1B). The FH of needles by CF ranged between $-29\text{ }^{\circ}\text{C}$ and $-46\text{ }^{\circ}\text{C}$ among the progenies. At T1, progenies U1 and F2 had the highest and lowest FH, respectively. FH decreased significantly between the two sampling times for all progenies except U1.

Shoot growth and biomass

At both T1 and T2, there were variations in new shoot length among the progenies at the same temperature (Fig. 2). In all progenies, a clear threshold for a decrease in new shoot length was observed between -8 and $-16\text{ }^{\circ}\text{C}$. In some progenies, new shoot length even increased between 4 and $-3\text{ }^{\circ}\text{C}$ at T1 before a decrease between -3 and $-8\text{ }^{\circ}\text{C}$, especially in the progenies U3 and F1. The most evident decline in the new shoot length took place in all progenies between -8 and $-16\text{ }^{\circ}\text{C}$. In the control samples ($4\text{ }^{\circ}\text{C}$), progeny F1 had the highest and U1 had the lowest new shoot length. The new shoot length was influenced by the sampling time, progeny, freezing temperature, and their interactions, including the interaction of time and temperature (Table 2). In

addition, all these factors significantly affected total shoot dry mass, except the interaction between the sampling time and progeny.

Root morphology

Differences in total root length between progenies were observed at both sampling times depending on the exposure temperature (Fig. 3). The most changes were found between -8 and $-16\text{ }^{\circ}\text{C}$, but little to no changes were observed between -16 and $-30\text{ }^{\circ}\text{C}$. At T1, root length had already decreased between -3 and $-8\text{ }^{\circ}\text{C}$ in progenies U1, U2, and U3. The longest and shortest root lengths in the control samples were observed in progenies U1 and U3, respectively (Fig. 3A). At T2, root length already decreased between 4 and $-3\text{ }^{\circ}\text{C}$ in all progenies except F1. The longest and shortest root length among the control samples were observed in progenies U1 and F1, respectively (Fig. 3B). Root length was significantly affected by sampling time, progeny, temperature, and their interactions, except the interaction of sampling time and progeny (Table 2).

The number of root tips decreased with decreasing freezing temperature in all progenies at both test times (Fig. 4). At T1, progenies U1 and U3 showed a decrease in the number of root tips at temperatures between -3

Fig. 1 Frost hardiness (FH) of needles of first-year Scots pine progenies from open-pollinated seed orchards in Ukraine (U1, U2, and U3) and in Finland (F1, F2, and F3) as assessed by the relative electrolyte leakage method (REL) (A) and by dark-acclimated chlorophyll fluorescence (F_v/F_m) (B). FH was tested twice (T1, T2) in one-month intervals during the cold acclimation in controlled conditions. Capital letters indicate significant differences between the progenies by frost hardiness tests in T1, and lower-case letters indicate the same in T2 ($P \leq 0.05$). The difference in FH between T1 and T2 within the same progeny is indicated by “*” ($P \leq 0.05$). No letter means no difference. Bars indicate the standard errors (REL, $n = 12$; CF, $n = 9$)

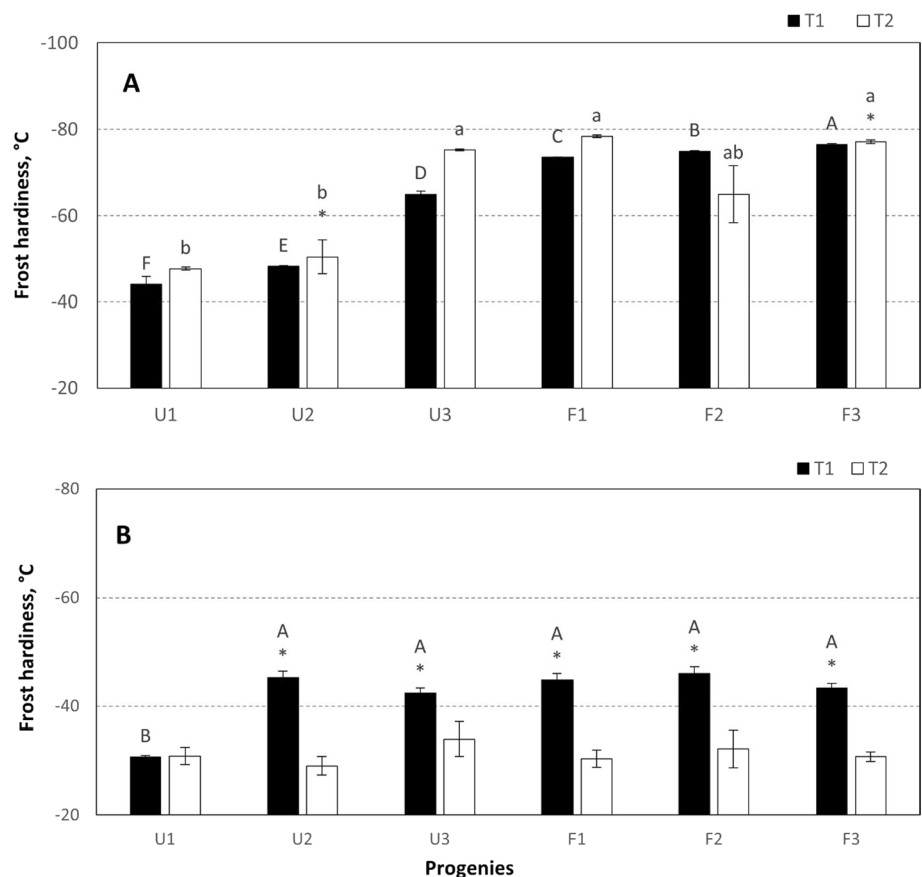


Fig. 2 The mean new shoot length of the first-year Scots pine seedlings of different provenies that were raised for three weeks in a greenhouse after exposure to different temperatures in one month intervals (test T1 and T2 in Figs. A and B, respectively) during cold acclimation in controlled conditions. The seedlings originated from the open-pollinated seed orchards in Ukraine (U1, U2, and U3) and Finland (F1, F2, and F3). Lower-case letters indicate the differences between provenies within the same temperature ($P \leq 0.05$). No letter means no difference. Bars indicate standard error ($n = 6$)

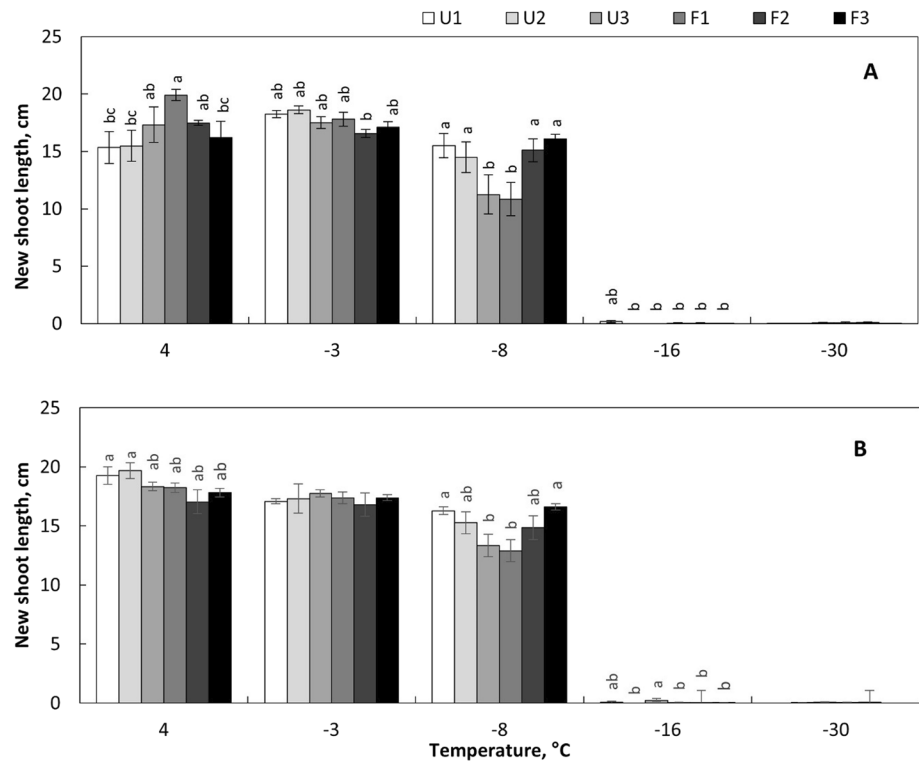


Table 2 The P -values for the source of variation of different variables by the sampling times (T) (T1 and T2), provenies (P) (U1, U2, U3, F1, F2, F3), and test temperatures (C) (4 °C, -3 °C, -8 °C, -16 °C, -30 °C), with their interactions (P -value ≤ 0.05 in bold)

Source of variation	New shoot length	Total shoot dry mass	Total root length	Number of root tips
Time (T)	< 0.001	< 0.001	< 0.001	0.607
Progeny (P)	< 0.001	< 0.001	< 0.001	< 0.001
Temperature (C)	< 0.001	< 0.001	0.002	< 0.001
T*P	0.299	0.566	< 0.001	0.043
T*C	< 0.001	< 0.001	< 0.001	< 0.001
P*C	0.183	0.030	< 0.001	0.003

and -8 °C, while all provenies showed a decrease at a temperature below -8 °C. The biggest and smallest number of root tips in the control samples (4 °C) were observed in provenies U2 and U1, respectively. At T2, a decrease in the number of root tips was already apparent between 4 and -3 °C, and a further decrease was observed between -8 and -16 °C, similarly as observed at T1. Differences in the number of root tips between provenies were observed at temperatures between -3 and -8 °C, with the biggest and smallest number of root tips in the control samples observed in provenies U1 and F1, respectively. The number of root tips was influenced by progeny, temperature, and their interactions, except for the sampling time (Table 2).

Frost hardiness between seed orchards in Finland and Ukraine by different methods

The comparison of two orchards (U vs F) by REL showed that the mean FH of needles was significantly higher in the Finnish than the Ukrainian orchard, the difference being 23 °C and 15 °C at the sampling times T1 and T2, respectively (Fig. 5, Table 3). However, there were no significant differences in FH between the sampling times or in the interaction between the sampling time and orchard (Table 3). The mean FH of needles by CF (F_v/F_m) was significantly lower at T2 than T1 in both Finnish and Ukrainian orchards (Table 3), but there were no differences between the orchards at either sampling time

Fig. 3 The mean total root length of the first-year Scots pine seedlings of different provenies that were raised for three weeks in a greenhouse after exposure to different temperatures in one-month intervals (tests T1 and T2 in Figs. A and B, respectively) during cold acclimation in controlled conditions. The seedlings originated from the open-pollinated seed orchards in Ukraine (U1, U2, and U3) and Finland (F1, F2, and F3). Lower-case letters indicate significant differences between provenies at the same exposure temperature ($P \leq 0.05$). No letter means no difference. Bars indicate the standard errors ($n = 6$)

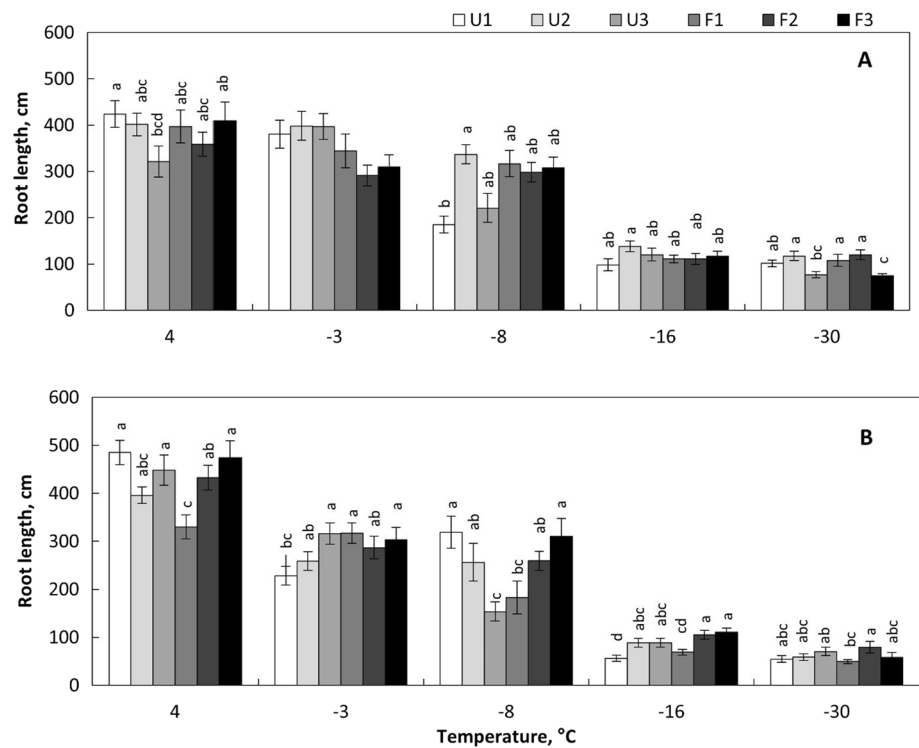
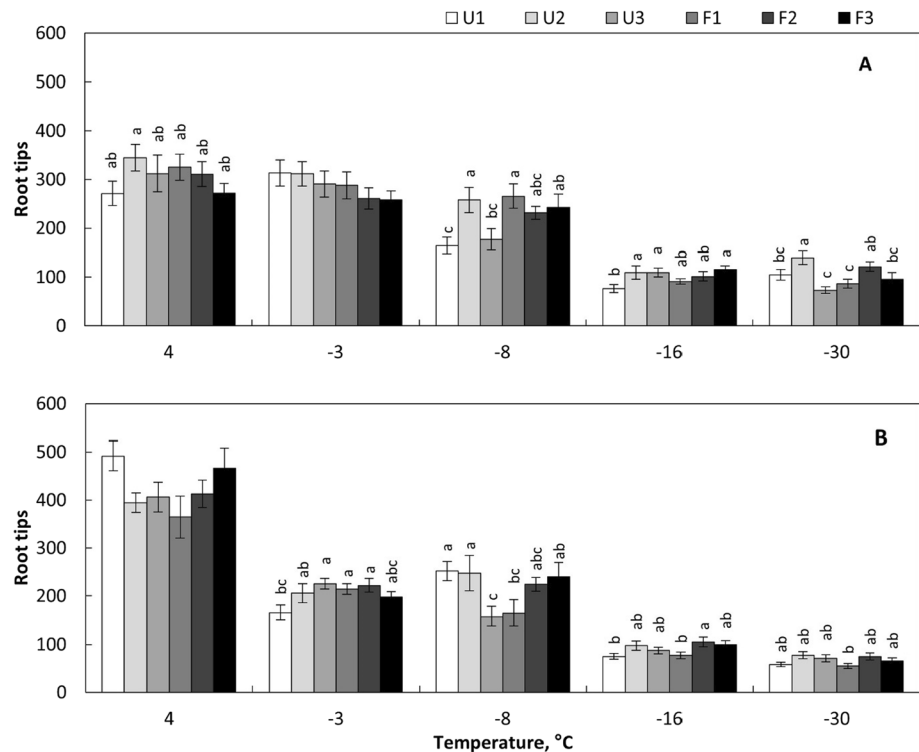


Fig. 4 The mean number of new root tips of the first-year Scots pine seedlings of different provenies that were raised for three weeks in a greenhouse after exposure to different temperatures in one-month intervals (tests T1 and T2 in Figs. A and B) during cold acclimation in controlled conditions. The seedlings originated from the open-pollinated seed orchards in Ukraine (U1, U2, and U3) and Finland (F1, F2, and F3). Lower-case letters indicate significant differences between provenies by test temperatures ($P \leq 0.05$). No letter means no difference. Bars indicate standard errors ($n = 6$)



(Fig. 5, Table 3). An FH assessment based on the length of the new shoot or the total length of the roots revealed on differences in the mean FH between the orchards and sampling times. The mean FH based on the number of root tips was higher at T2 than T1. However, there was no

difference in mean FH between the orchards. The mean FH based on total shoot dry mass was affected by the sampling time (Table 3).

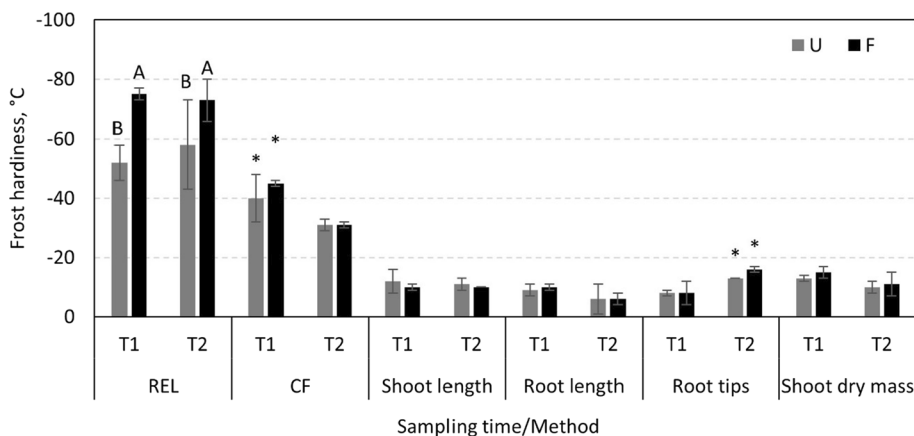


Fig. 5 The mean frost hardiness (FH) of the three progenies in the seed orchards in Ukraine (U) and Finland (F) at two samplings times (T1 and T2) as assessed by relative electrolyte leakage (REL) and chlorophyll fluorescence (CF) of needles, shoot length, root length, the number of root tips, and shoot dry mass. Different capital letters

indicate the statistically significant difference between the orchards at T1 and T2, no letters mean no difference. The asterisk “*” indicates the statistically significant difference between two sampling times within the same orchard ($P < 0.05$). Bars indicate standard errors ($n = 3$)

Table 3 The significance of the difference (P -value) in the mean frost hardiness (FH) of Scots pine seedlings of two seed orchards (O) in Ukraine and Finland at two sampling times (T) as assessed by the

relative electrolyte leakage (REL) and chlorophyll fluorescence (CF) of needles, by shoot length, root length, the number of root tips, and total shoot dry mass (P -value ≤ 0.05 in bold)

Sources of variation	FH assessment method					
	REL	CF	New shoot length	Root length	Root tips	Total shoot dry mass
Time (T)	0.751	0.002	0.591	0.063	0.001	0.035
Orchard(O)	0.011	0.329	0.199	0.921	0.326	0.383
T*O	0.574	0.291	0.591	0.766	0.433	0.508

Discussion

Frost hardiness of needles

In contrast with our hypothesis, the FH of the needles as assessed immediately after the freezing tests by the REL method was higher in the seedlings originating from the Finnish orchard than in those coming from the Ukrainian orchard. This was the case even though the same plus tree genotypes with the same mother trees were cultivated from the grafts. The seed orchards were more than 1600 km apart. Their light and temperature conditions therefore differed greatly, but due to the grafts, there was no opportunity for local adaptation. However, as an epigenetic effect, there is a possibility of local adaptation in seed development as in the development of pollen and embryos in the new environmental conditions (Eriksson et al. 1980; Beuker 1994; Persson 1994; Lehtinen and Pulkkinen 2017; Alakärppä et al. 2019). The memory of environmental

conditions across generations can impact the acclimation potential, which may be manifested in the physiological development of seedlings and affect their FH as well (Bose et al. 2020). It is known that growth in diverse environmental conditions is possible through plastic responses, genetic differentiation, or epigenetic inheritance (Johnsen et al. 1996; Skråppa et al. 2007), which was also supported by our results for FH. In the seed orchards in Finland, a southern effect may lead to a minor decrease in FH due to the southern winds at the time of pollination (Johnsen et al. 1996; Lehtinen and Pulkkinen 2017; Chan 2019). However, we did not observe this effect in the offspring of the Ukrainian seed orchard. This could be because in Finland the plus trees began pollination seven to ten days earlier than in Ukraine, making cross-pollination between the two populations unlikely (Pulkkinen et al. 1995; Hayda et al. 2019; Neyko et al. 2020). As the cold acclimation conditions were similar for all the progenies during the two sampling times, we can conclude that the differences in the FH of needles between the progenies were due to

other than environmental factors, e.g., epigenetic factors (Lehtinen and Pulkkinen 2017; Andersson Gull et al. 2018; Neimane et al. 2018; Alakärppä et al. 2019; Chan 2019; Bose et al. 2020).

The frost hardiness of needles by chlorophyll fluorescence was much lower than the FH assessed by REL. The difference varied between 3 and 34 °C, depending on the progeny. In addition, frost hardiness by CF tended to decrease between sampling times, i.e., with prolonged maintenance of the seedlings in cool conditions, which was not found by REL. Because the FH assessment by CF is based on the efficiency of the electron transfer chain of *PSII* in chloroplasts, whereas REL is a measure of the ion leakage of plasma membranes, the differing results for FH by CF and REL of needles were due to different cellular processes in freezing stress.

Except for U2 and F3, there were no significant changes in the FH of the needles between sampling times by REL. However, the FH of needles was already quite high across all progenies at the first sampling time. Before that test, the seedlings had been maintained for ten weeks at a cool temperature with a short photoperiod. Both are the key drivers of cold acclimation of aboveground organs in the early phase of cold acclimation (Weiser 1970; Christersson 1978). It is possible that the FH was already close to a steady state, corresponding to the conditions before sampling time T1 (Leinonen et al. 1995). As we did not start monitoring the FH from the beginning of the cold acclimation, we cannot conclude whether there was a time difference between the progenies at the start of cold acclimation. In the previous studies, clear differences in the initiation of frost hardening have been found between Scots pine genotypes (Hurme et al. 1997; Repo et al. 2000; Nilsson 2001).

Frost hardiness based on root and shoot growth

The frost hardiness based on the growth of roots and shoots, as assessed after the growth test, was much less than FH by REL and CF of needles immediately after the freezing tests. According to the shoot and root growth, FH varied from – 10 to – 15 °C and from – 6 to – 16 °C, depending on the trait used in the FH assessment, without differences between the progenies for either shoots or roots, respectively. In contrast with a previous study with freeze-stored containerized seedlings of Scots pine (Di et al. 2019), we did not observe new root tip formation in the seedlings exposed at low temperatures, which indicated root damage. The whole-plant freezing test followed by the raising of the seedlings in favorable conditions integrated the injuries in different organs, with roots as the most sensitive plant part (Bigras et al. 2001). In our study, roots determined the whole-plant FH of the seedlings, and therefore explained the differences in FH as assessed immediately after the freezing tests and after the

growing periods in the greenhouse. In field conditions, roots are not exposed to temperatures as low as shoots are, and their effects on whole-plant FH would therefore not be as decisive as in our study. However, in accordance with our hypothesis, we can conclude that there were no differences in the FH of roots between the orchards and progenies.

The conditions prior to the first freezing test were favorable for bud development, and the buds were not therefore expected to be susceptible to frost. Bud injuries would not explain the needle damage or the retarded growth of roots either. Previously, the stem was found to be less frost hardy than the needles (Ryypö et al. 1998; Repo et al. 2000), which may have been the case in our study too, even though they were not tested separately. The most probable explanation for the inhibited growth of shoots and roots is the damage in the roots or root collar, which therefore impeded water and nutrient uptake and resource transport between the roots and shoots.

Conclusion

This study's objective was to investigate the differences in the FH of Scots pine seedlings that originated from the same plus tree progenies in seed orchards in Finland and Ukraine during cold acclimation. Our results showed that the frost hardiness of needles, as assessed immediately after the freezing tests, was higher in the Finnish than in the Ukrainian seed orchard progenies. However, when the seedlings were grown in the greenhouse after the whole-plant freezing tests, and their FH was assessed based on the growth of shoots and roots, the differences between progenies as observed for needles disappeared. This suggests that there were no significant differences in the FH of roots between seed orchards and progenies. In the field, with snow cover, roots are typically not exposed to temperatures as low as shoots in midwinter, and their effects on FH and growth would therefore be less decisive, as inferred from the results of this study. In conclusion, our findings suggest that seed produced in Southern Ukraine poses a low risk to use for practical reforestation actions in Southern Finland.

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Authors contributions Conceptualization (DW, TR, PP), methodology (DW, TR, PP), measurements (DW), data curation and statistics (DW, TR, JH), writing – original draft preparation (DW, PP, AP, IN, GZ, BD, JH, TR), resources (TR, PP, IN), supervision (TR, PP, AP, GZ), writing – reviewing and editing (DW, PP, AP, IN, GZ, BD, JH, TR).

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Data availability The data of this study are available from the corresponding author (DW) on request.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no conflict of interest.

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