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Genetic Variation among Somatic Embryo Clones of Nordmann Fir Grown as Christmas Trees

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Abstract: Somatic embryogenesis (SE) cloning techniques for Nordmann fir Christmas tree production have been pursued and refined in Denmark, and three SE clonal trials with 201 SE clones from seeds of 27 Nordmann fir trees and seedlings from bulk harvest seeds in Georgia, Caucasus, were established from 2014 to 2015. This study was the first to evaluate the genetic performance of these SE clones in different sites. In addition, the performance of SE clones and (zygotic) normal seedlings in the field was compared in this study. This study was based on three key groups of traits for Christmas tree production, measured 5 and 6 years after planting: growth, commercial tree quality, and disorder. Seedlings grew faster and had better Christmas tree quality than SE clones. There were significant family effects for all traits, indicating that a good family also produced good clones on average. Growth and disorder traits were under moderate genetic control, with estimated broad-sense heritability (H^2) from 0.19 to 0.31 and from 0.20 to 0.28, respectively. Quality traits had low to moderate H^2, ranging from 0.09 to 0.24. Significant genotype-by-environment interactions were shown for Christmas tree quality, post-harvest needle retention, and branch angle, suggesting the importance of choosing the right clone at each site. There was no significant interaction in height among the years. Superior clones, compared to the unbred seed source, were identified by combining several important traits. Overall, the somatic clones were well established but had slower growth compared to standard seedlings.

Keywords: *Abies nordmanniana*; somatic embryogenesis; heritability; Christmas tree; clone

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1. Introduction

Christmas trees are widely used in many countries due to their traditional usage and are an important economic product [1–3]. Different tree species are used as Christmas trees in different parts of the world. Nordmann fir (*Abies nordmanniana* (Steven) Spach) is native to the Caucasus mountains and is the most frequently used Christmas tree in Europe due to its beautiful dark green color, long soft needles, and good needle retention [4].

Denmark has been one of the main European producers of Nordmann fir Christmas trees since the 1990s. A Danish Christmas tree improvement program was established in 1992, and within this framework, selection and breeding of a wide range of genotypes have been carried out for over 30 years. Now, the breeding program is at the beginning of the second generation [5–8]. About a 22% gain has been attained through the first-generation breeding process for improving Christmas tree quality [5]. It is well-established industry knowledge that roughly 10%–40% of Nordmann fir trees are discarded because of their failure to achieve the desired characteristics when grown to a full-size Christmas tree after 8 to 10 years in the field. Therefore, it has been expected that if genetically selected elite and stably performing Christmas trees could be cloned, it would improve the percentage of trees sold per hectare and growers' revenue.

Somatic embryogenesis (SE) is the process that embryos developed from vegetative tissues, rather than from the zygote. The obtained embryos are clones of the plant from which

the vegetative tissues were taken, and they strongly resemble zygotic embryos in seeds. Since the 1980s, conifer SE has been recognized as an advanced vegetative propagation technology [9–11] which allows for the long-term preservation and mass production of elite genotypes. Since the 1990's, efficient SE cloning techniques for Nordmann fir Christmas tree production have been pursued at the University of Copenhagen [12,13]. Find [14] described the first results from a demonstration planting of 9 clones and documented the viability and growth of Nordmann fir SE clones after 6 years in the field and scheduled plans for further implementation. The SE methods were refined by a research group at the University of Copenhagen, resulting in a method patent [15], and recently, results from nursery growth of SE clones related to the specific steps of the SE plant production procedure were presented by Nielsen et al. [16]. SE clones of Nordmann fir in Germany have also been described by Blödtner-Piske [17].

Understanding the genetic control and variation in SE clones and their relationship with the planting environment is a fundamental step to integrating SE technologies into traditional genetic improvement programs [18–20]. Several studies have shown significant clonal variations and stable clone performance for growth across different environments [19,21]. There were pronounced genotype-by-environment (G × E) interactions involved in *Pinus taeda* SE clones, as determined by a genetic analysis at four sites with measurements at three ages [20]. Few studies have been conducted to evaluate the field performance of SE clones, and no difference has been observed between SE and zygotic seedlings in physiological or morphological performance [22–24]. In contrast, significant differences have been observed in white spruce (*Picea glauca* (Moench) Voss) [25]. However, studies have generally been based on a small number of families or only been observed in the greenhouse, often at a very early stage of development, leading to inaccurate estimates of variance; thus, conclusions must be drawn with caution. Currently, no studies have evaluated genetic parameters related to field performance for Nordmann fir SE clones.

Comparing traditional temperate forestry and Christmas tree production, the latter has a short rotation (max. 10 years), makes extensive use of fertilizers and weed and pesticide control, and views the tree itself as the final product. The price of a sold Christmas tree is generally based on height, quality, and downgrading due to damage [26]. Therefore, major characteristics of interest are grouped into three categories: (1) growth traits including height, width of tree indicated by the branch length at the 1st whorl, and growth vigor represented by the number of branches and branch angle at the 1st whorl; (2) quality traits, including Christmas tree grade, needle color, and post-harvest needle retention. The latter is a major factor for consumers but is not directly included in the price setting; (3) disorder traits include any tree disorder influencing visual appearance. Three SE clonal trials were established in Denmark from 2014 to 2015, which gave us the opportunity to evaluate the SE clones with the objectives to: (1) evaluate SE clones' performance compared with zygotic seedlings; (2) estimate family performance and clonal genetic parameters as genetic variance and broad sense heritability; and (3) evaluate clone stability, measured as genotype-by-site, and year-by-year interactions of different traits related to Christmas tree production.

2. Materials and Methods

2.1. Plant Material and Test Sites

Embryogenic cultures of *A. nordmanniana* were initiated from zygotic embryos, derived from seeds collected from 27 trees in the Ambrolauri region in Georgia, Caucasus (Figure 1; Table S1). The selected trees were clustered into three areas near the village Agara (NW) east of Nikortsminda, Tskadisi (NE), and a cluster of trees at the very northeasterly part of the Shaori water reservoir (SW). All three areas are part of the Ambrolauri Tlugi Forest district departments 2, 3, and 16BS, respectively. The methods used for the establishment of embryogenic cultures, cryopreservation, proliferation, maturation, and germination are similar to those described in the work of Find [15]. The embryogenesis process and plantlet regeneration has been further documented and illustrated by Nielsen et al. [16].

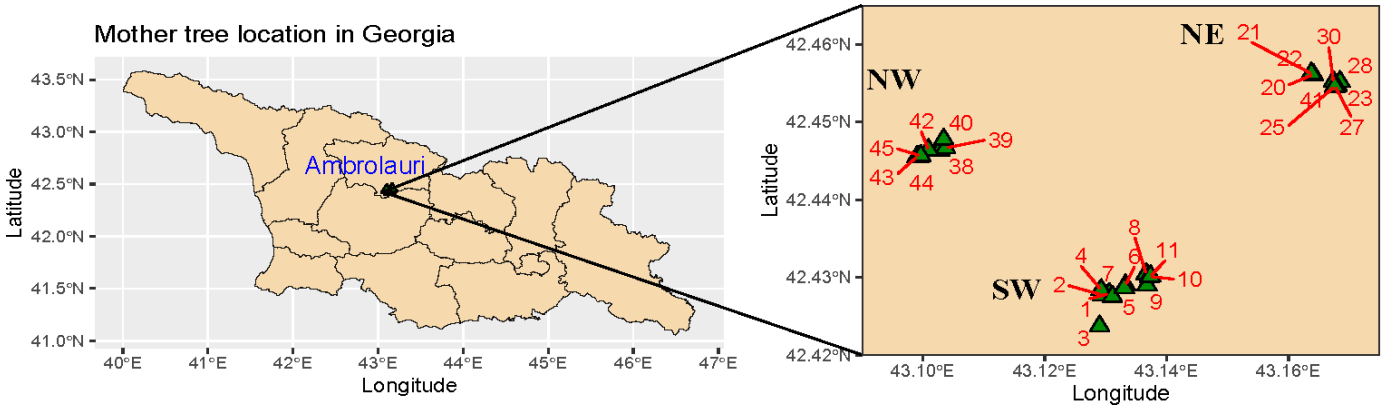

Figure 1. Geographical distribution of the 27 mother trees clustered into three areas (NE, NW, and SW) in the Ambrolauri region in Georgia, Caucasus. Each red number is the label of the 27 mother trees.

In autumn 2014 and 2015, three trials were established in Denmark from 201 clones (Figure 2), using a randomized block design with single-tree plots at a spacing of 1.1 m × 1.1 m to 1.5 m × 1.7 m between plants (Table 1). In general, there were 10 blocks. However, not all clones were available for planting across the three sites. Lundbygaard and Ry had 128 common clones, and Holstebro had 12 common clones between Lundbygaard and Ry. There were 11 common clones across all three sites. In Lundbygaard and Ry, in addition to SE clones, zygotic seedlings, extracted from a commercial bulk harvest of seeds in the same region that the mother trees were from, were also included in these field trials. In Ry, SE clonal trees were under-planted in an existing commercial stand, which acted as over-story for the SE clones during the first three years. This had the effect of partly improving microclimate, and partly of causing some extra competition. Some of the over-story trees were sold and removed every year, leaving more and more growing space for the SE plants.

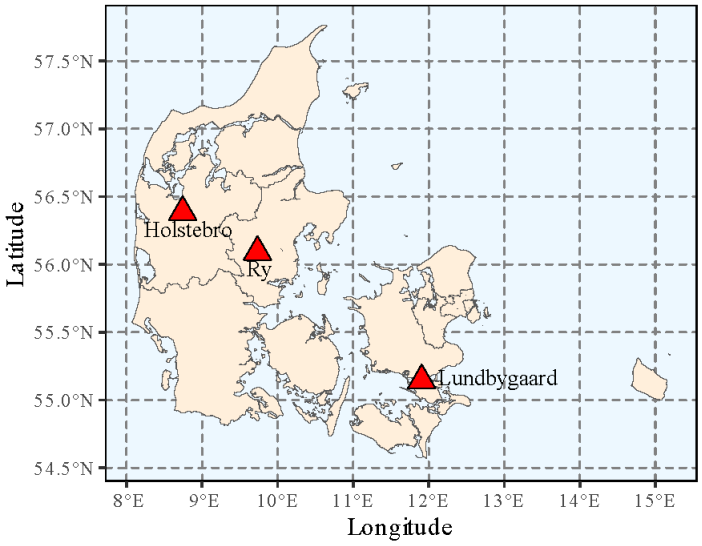

Figure 2. Location of the three SE clonal trials in Denmark.

2.2. Measurements

The measured traits were grouped into three categories: growth, Christmas tree quality, and disorders (Figure 3). Measurements were recorded after 6 growing seasons at Lundygaard and Ry and after 5 growing seasons for Holstebro. Christmas tree quality and bare shoulders were measured at Holstebro at the mark of 6 growing seasons.

Table 1. Summary of site information.

Site Information	Lundbygaard	Ry	Holstebro
Plant year	Autumn 2014	Autumn 2014	Autumn 2015
Soil type	Moraine	Meltwater sand	Meltwater sand
Spacing	1.5 m × 1.7 m	1.1 m × 1.1 m	1.1 m × 1.15 m
No. of clones	153	129	62
No. of half-sib families	25	25	23
Average No. clones/family	6.1	5.2	2.6
Range no. clones per family	1–18	1–17	1–6
Average No. of ramets/clone	10.8	8.6	9.4
Range of No. ramets/clone	1–72	2–15	5–20
Row/Column	38/52	16/100	6/243
No. SE trees	1636	1124	574
No. seedlings	153	122	0

Figure 3. Photos describing the traits: (**A**) Christmas tree quality; (**B**) branch angle; (**C**) needle disorder: bare shoulders; (**D**) post-harvest quality; (**E**) needle color.

2.2.1. Growth

- The cumulative final heights of the years 2016, 2017, 2018, 2019, and 2020 were obtained after 2, 3, 4, 5, and 6 growing seasons, respectively; thus, the annual height increments of the years 2017, 2018, and 2019 were recorded.
- The length of the current year's new shoot growth at the uppermost whorl was measured.
- The number of branches in the uppermost whorl, which is an important characteristic reflecting tree quality and also related to growth vigor, was counted.
- The angle of branches was compared to the main stem in the uppermost whorl on a scale from 0 to 9 (0 = flat, ca. 0 degrees; 9 = vertical, ca. 90 degrees).

2.2.2. Christmas Tree Quality

- Christmas tree quality was scored on a scale from 1 (worst) to 9 (best), following the methods outlined by Nielsen et al. [7].
- Needle color was scored into categories in steps of 0.5, from pale yellow, score 1, to very dark green, score 4.
- Post-harvest needle loss of twigs was observed indoors for 10 days. One twig from a side branch was harvested on all trees in mid-November—after 6 growing seasons for Lundygaard and Ry and 5 seasons for Holstebro. Following the procedures developed by Nielsen and Chastagner [4], the twigs were immediately transported to an indoor testing facility, where they were kept for 10 days at 20 °C with a relative humidity of around 55%. After gently stroking each twig between two fingers in the direction from base to tip, needle loss was evaluated on a scale from 0–7, where 0: no needle loss, 1: <1%, 2: 1%–5%, 3: 6%–15%, 4: 16%–33%, 5: 34%–66%, 6: 67%–90%, and 7: 91%–100%.

2.2.3. Disorders

- Bare shoulders were measured as needle loss or yellowing of last year's needles in the second uppermost whorl of the tree. Score 0: none; score 1: yellowing of needles and minor needle loss; scores 2 and 3: moderate and severe needle loss, respectively.
- Needle loss due to unknown reasons for branches seen in the field was bivariate.

2.3. Statistical Data Analysis

An analysis of variance (ANOVA) was performed in ASReml 4.2 [27], where site and group were fitted as fixed effects for all studied traits to evaluate the difference in field performance between seedlings and SE clones. Wald F statistics were used to test the significance of each fixed effect. The best linear unbiased estimates, equal to the least square mean (LSmean) for groups of each fixed effect, were obtained.

The cross-site analysis for all measured traits in the three clone trials used the following model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{e} \quad (1)$$

where $\mathbf{y}$ is the combined vector of individual phenotypic values of the response variables in three clonal trials, $\mathbf{b}$ is the combined vector of fixed effects (i.e., grand mean, site, block within site effect, mother tree distribution area, family, family within site effect), and $\mathbf{u}$ is the combined vector of the random clone effect and clone-by-site interaction effect. $\mathbf{X}$ and $\mathbf{Z}$ are the known incidence matrices related to $\mathbf{b}$ and $\mathbf{u}$, respectively, and $\mathbf{e}$ is the combined vector of residuals from each site. The random effects were assumed to follow a multivariate normal distribution, with means and variances defined as $\mathbf{u_c} \sim \mathbf{N}(\mathbf{0}, \mathbf{I}\sigma^2_{\mathbf{c}})$, $\mathbf{u_{cs}} \sim \mathbf{N}(\mathbf{0}, \mathbf{I}\sigma^2_{\mathbf{sc}})$, and $\mathbf{e} \sim \mathbf{N}(\mathbf{0}, \mathbf{I}\sigma^2_{\mathbf{e}})$, where $\mathbf{0}$ is the expectation; $\sigma^2_{\mathbf{c}}$ is clone variance across sites, $\sigma^2_{\mathbf{sc}}$ is variance of clone-by-site interaction, $\sigma^2_{\mathbf{e}}$ is the residual variance at each site, and $\mathbf{I}$ is the identity matrix.

Assumptions of normality and variance homogeneity were accepted for all traits based on the visual inspection of residuals against predicted value plots for each trait.

For binary trait needle loss, a logit link function was used to fit in the Equation (1) to estimate variance components:

$$\mathbf{y} = \mathbf{log}\left(\frac{\mathbf{p}}{\mathbf{1-p}}\right) = \mathbf{X\beta} + \mathbf{Zu} + \mathbf{e} \quad (2)$$

where y is the link function $\mathbf{log}\left(\frac{\mathbf{p}}{\mathbf{1-p}}\right)$, p is the proportion of trees with needle loss score = 1.

In addition, a correlation **(CORGH) G** structure was used to directly estimate the type-B genetic correlation (r_{bg}) between sites:

$$\textbf{CORGH G structure} = \begin{bmatrix} \sigma^2_{c1} & \rho_{12} & \rho_{13} \\ & \sigma^2_{c2} & \rho_{23} \\ & & \sigma^2_{c3} \end{bmatrix} \quad (3)$$

where ρ_{12} and ρ_{13} estimate r_B between sites Holstebro with Ry and Lundbygaard, respectively, ρ_{23} estimates r_B between sites Ry and Lundbygaard, and σ^2_{c1}, σ^2_{c2} and σ^2_{c3} estimate the variance components for SE clones at each site.

2.4. Genetic Parameters

Individual broad-sense heritability (H^2) was estimated across sites as follows:

$$H^2 = \frac{\sigma^2_c}{\sigma^2_c + \sigma^2_{sc} + \sigma^2_{\frac{-}{e}}} \quad (4)$$

where $\sigma^2_{\frac{-}{e}}$ is the average within-site residual variance. Standard errors were estimated using ASReml.

Best linear unbiased predictions (BLUP) of the genetic values of clones were obtained from the predicted values file from ASReml and adjusted using the fixed family effect.

3. Results

3.1. Zygotic Seedlings vs. SE Clones

There were 275 zygotic seedlings and 2760 SE plants at Lundbygaard and Ry sites. ANOVA showed that the four traits were significantly different between zygotic seedlings and SE plants (Table 2). Zygotic seedlings grew faster than SE clones, had more twigs at the 1st whorl, and displayed slightly better Christmas tree quality. Increment growth showed that zygotic seedling growth was faster every year than the SE clones (Figure 4). The increment in growth from 2017 to 2020 clearly showed reduced growth in 2018 and 2019, which was due to the record drought in Europe. Zygotic seedlings grew slightly more than SE clones during the drought year.

Table 2. LSmean of zygotic seedling and somatic embryogenesis (SE) clones group for traits: branch length at 1st whorl; number (No.) of twigs at 1st whorl; total height of tree; and Christmas tree quality score.

	Branch Length 1st Whorl/cm	No. Twigs 1st Whorl	Height/cm	Christmas Tree Quality/Score
p value	<0.001	<0.001	<0.001	0.03
Seedling	24.9 (0.37)	5.1 (0.10)	122.8 (1.80)	3.4 (0.09)
SE clones	23.0 (0.12)	4.5 (0.03)	102.0(0.59)	3.2 (0.03)

3.2. Site Means and Family Effect

Overall, trees were measured at ages 5–6 years old in the fields. Multi-site analysis was conducted for all common traits across the three sites, and there was a significant site effect for all traits (Table 3). Trees grew slowest in Ry compared with the other two sites, even though the trees in Ry were one year older than those in Holstebro. For quality traits, the most obvious difference was in Christmas tree quality score, where Ry had on average the worst Christmas tree quality score (LSmean 2.86 se: 0.08), and Holstebro and Lundbygaard had on average similar Christmas tree quality scores (Average LSmean: 3.55). In addition, post-harvest needle loss showed significant differences at different sites. Finally, Ry had the least disorder problems.

There was also a significant family effect for all studied traits, although the distribution of the number of clones per family was skewed. Based on the estimated BLUPs of clones in each family across the different traits, some families performed much better than others, showing a pattern in which a good family also produced good clones on average (Figures 5 and 6). For example, families 39 and 1 had moderate growth, superior Christmas tree quality, and very few needle disorder issues due to bare shoulders. Furthermore, family 39 had excellent post-harvest needle retention, which was not the case for family 1.

Although the distance between the three geographical locations of the 27 mother trees was only from 3 to 5.5 km, a significant difference was detected for five traits (Figure 7). Mother trees from SW performed the worst due to flat branches, low Christmas tree quality, high post-harvest needle loss, and high rate of bare shoulder disorder problems. The other two areas performed generally better in most traits.

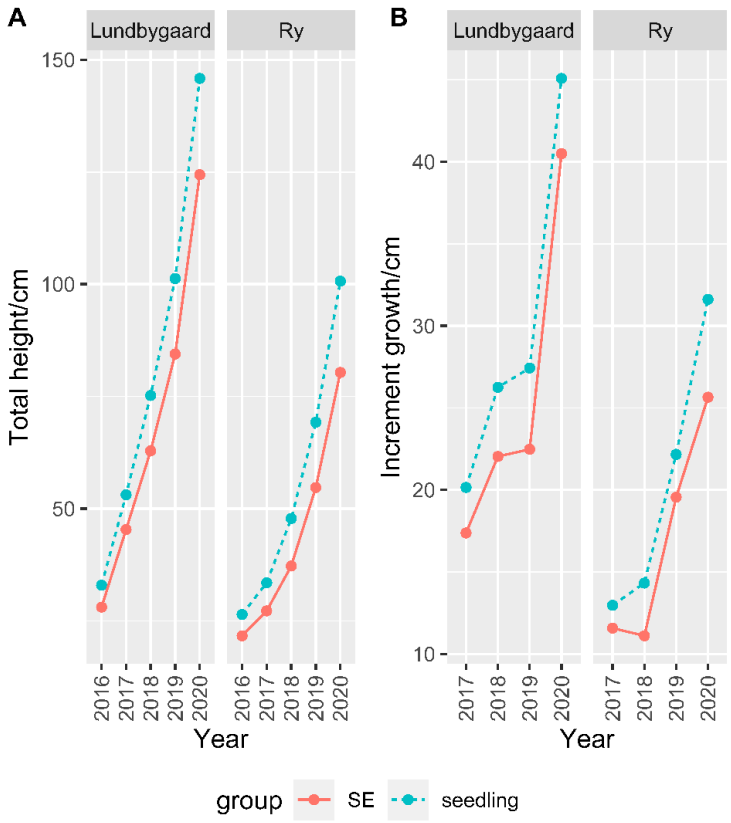

Figure 4. (**A**): Mean height between somatic embryogenesis (SE) and zygotic seedlings (seedling) from 2016 to 2020 at Lundbygaard and Ry; (**B**): Mean increment growth between SE and seedlings from 2017 to 2020 at Lundbygaard and Ry.

Table 3. LSmean and genetic parameters for multi-sites analysis of all studied traits.

	Field Age	*p* Value of Fixed Effects		LSmean of Each Site			Genetic Parameters		
		Site	Family	Hol	Ry	Lund	σ_c^2	σ_{sc}^2	H^2
Growth traits									
Height	5–6	<0.001	<0.001	87.3 (8.69)	72.8 (8.51)	123.8 (8.76)	228.55 (32.98)	43.49 (11.66)	0.31 (0.03)
Branch length 1st whorl	5–6	<0.001	0.03	18.2 (0.39)	19.1 (0.33)	25.9 (0.34)	5.58 (0.95)	1.42 (0.48)	0.19 (0.03)
Number of branches	5–6	<0.001	<0.001	4.8 (0.09)	4.5 (0.09)	4.4 (0.07)	0.27 (0.05)		0.10 (0.02)
Quality									
Christmas tree quality	6	<0.001	<0.001	3.5 (0.10)	2.8 (0.08)	3.6 (0.08)	0.17 (0.05)	0.16 (0.04)	0.09 (0.02)
Branch angle	5–6	<0.001	<0.001	3.7 (0.13)	3.5 (0.11)	4.0 (0.11)	0.60 (0.10)	0.18 (0.05)	0.24 (0.03)
Needle color	5–6	<0.001	<0.001	1.6 (0.03)	1.6 (0.03)	1.9 (0.03)	0.03 (0.01)	0.01 (0.003)	0.15 (0.02)
Post-harvest needle retention	5–6	<0.001	<0.001	0.6 (0.08)	0.5 (0.06)	0.8 (0.06)	0.11 (0.03)	0.12 (0.03)	0.11 (0.03)
Disorders									
Bare shoulders	6	<0.001	0.01	0.7 (0.08)	0.4 (0.07)	0.7 (0.07)	0.26 (0.04)	0.07 (0.02)	0.28 (0.03)
Needle loss	5–6	<0.001	0.009	0.6 (0.03)	0.5 (0.03)	0.5 (0.03)	0.05 (0.01)	0.004 (0.003)	0.20 (0.03)

Notes: Hol: Holstebro; Lund: Lundbygaard; σ_c^2: clone variance across sites; σ_{sc}^2: variance of clone-by-site interaction; H^2: individual broad-sense heritability.

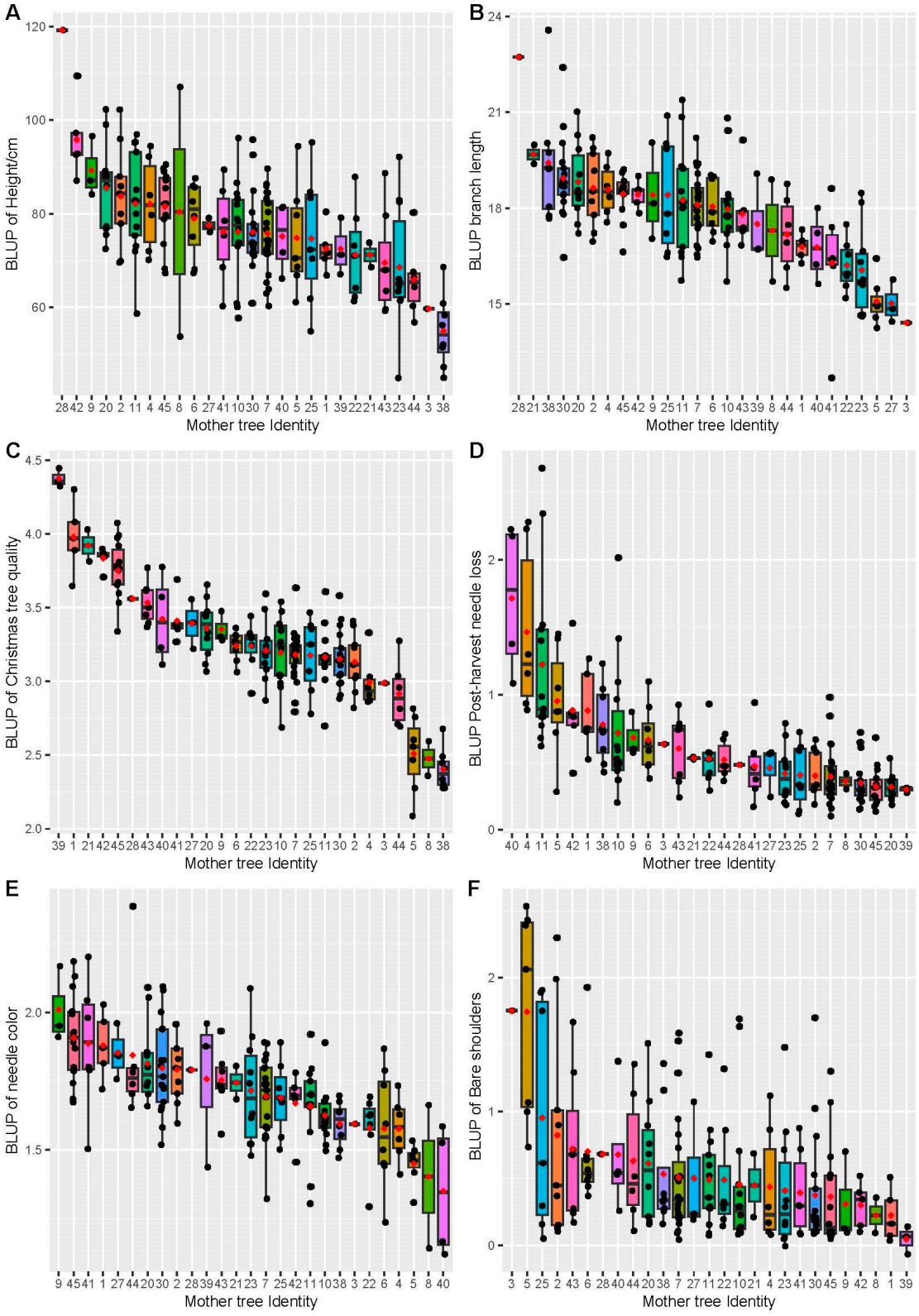

Figure 5. Boxplot of best linear unbiased predictions (BLUPs) of 27 half-sib families for studied traits; the red dot in each box indicates the mean BLUP of each half-sib family; black dots around each box indicate clone values of clones from each half-sib family. Traits: (**A**) height, (**B**) branch length; (**C**) Christmas tree quality; (**D**) post-harvest needle loss; (**E**) needle color; and (**F**) needle disorder bare shoulders.

Figure 6. Photos showing naturally grown clones in demo-planting at Holstebro site after 6 growing seasons. (**A**) The image shows a standard, well-performing clone, and shows in front a slow-growing clone of poor quality. (**B**) Good-performing clone 42.02 after 7 seasons.

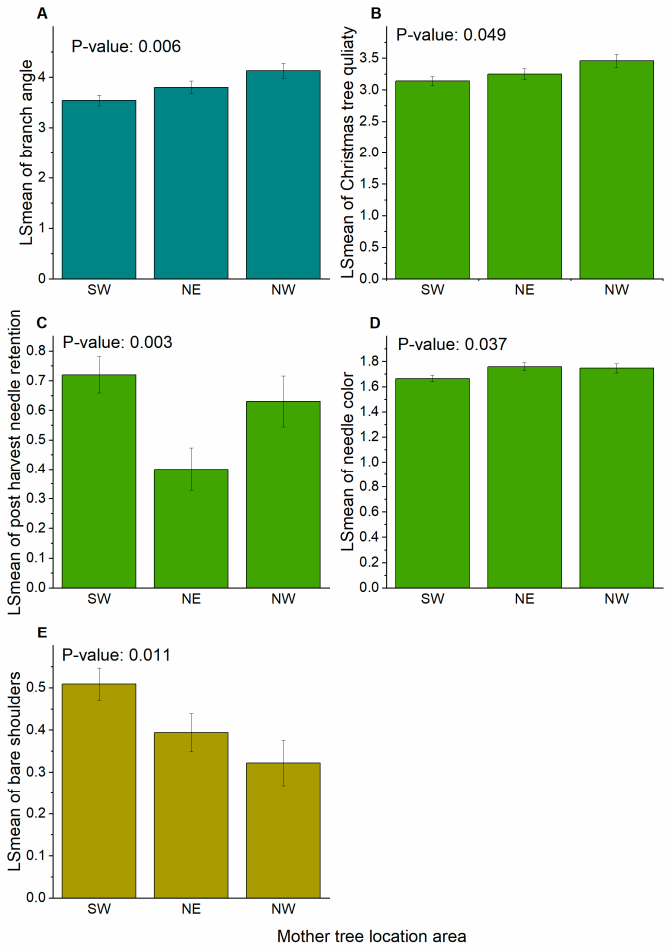

Figure 7. LSmean of the three mother tree location areas (SW, NE, NW) for (**A**) branch angle; (**B**) Christmas tree quality; (**C**) post-harvest needle retention; (**D**) needle color; (**E**) bare shoulders. The three colors indicate growth, quality, and disorder categories, respectively.

3.3. Variance Components, Heritability, and Stability

Overall, pronounced clonal variation was detected for all studied traits (Table 3). Based on the estimated broad-sense heritability (H^2), in general, growth traits were under

moderate genetic control (0.19 to 0.31), except for the number of branches (0.10). Quality traits had low to moderate H^2, from 0.09 to 0.24, and disorder traits had moderate H^2 from 0.20 to 0.28.

Significant family-by-site effects were only shown for Christmas tree quality (*p* value: 0.009) (Figure 8). Ranking families based on the estimated LSmean and comparisons among sites showed that family rank changed at different sites.

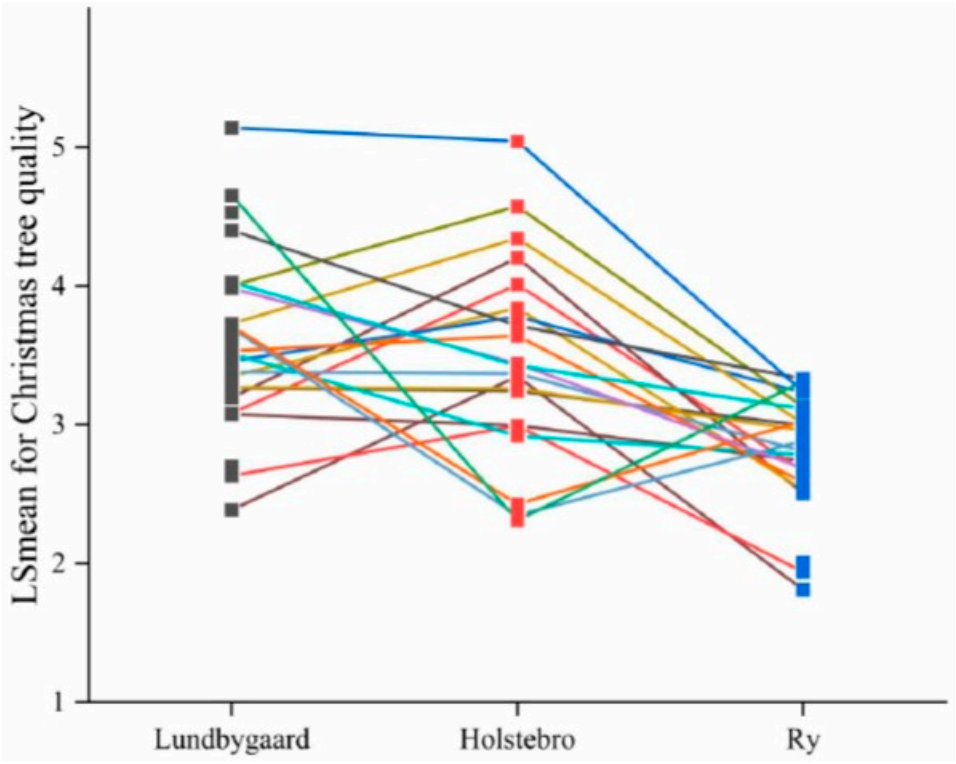

Figure 8. LSmean of Christmas tree quality score for families at different sites. Each line indicates one family.

For all traits, clones showed significant clone-by-site interactions, except in the case of the number of branches at the 1st whorl, although, for most traits, the estimated interaction variation constituted less than 36% of the main effect of clones (Table 3). The variation due to clone and site interaction resembled the main clone variation only for the Christmas tree score and post-harvest needle retention. Clone performance across sites is shown in Figure 9.

Clonal stability was evaluated more specifically by estimating type-B correlations describing the degree of similarity between sites. Three traits had low type-B genetic correlation (r_{bg}) between sites, indicating very strong G × E interactions. For Christmas tree quality, there was no genetic correlation between Holstebro and the other two sites, while Lundbygaard and Ry had an r_{bg} of 0.59 (se: 0.13). Post-harvest needle retention showed that Lundbygaard had low r_{bg} at Ry (0.47 se: 0.11) and Holstebro (0.44 se: 0.13), while Ry and Holstebro had very high r_{bg} (0.92 se: 0.16). Branch angle results also suggested that there was less genetic correlation between Holstebro and other sites, while Ry and Lundbygaard had a quite high r_{bg} (0.89 se: 0.05)

Since height was measured for several years, the interaction of increment growth for each year was used to study the year-by-year interaction. There was no significant year-by-year interaction for increment growth across all three sites; the average genetic correlation between years was 0.82.

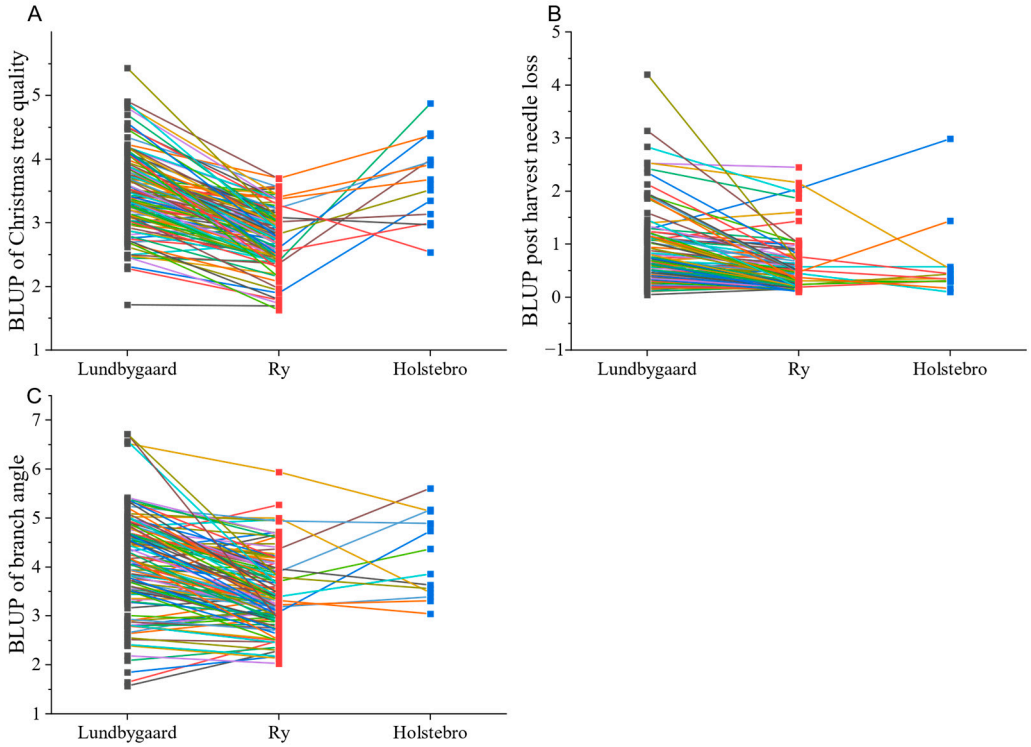

Figure 9. BLUP values for individual clones across sites for traits: (**A**) Christmas tree quality; (**B**) post-harvest needle retention; (**C**) branch angle. Different lines represent different clones.

4. Discussion

Overall, this study documented well-established and well-growing somatic plants of Nordmann fir, developing into Christmas trees at three different sites, and a large genetic variation between clones and families was detected. Both clone and family-by-site interactions were significant for the most important trait, Christmas tree quality.

The seed source from Ambrolauri, Georgia, has long been one of the preferred tree provenances throughout Europe, as outlined by Fløistad et al. [28]. Its superiority was first recognized based on provenance testing starting in the 1960s [29] and was generally characterized by moderate growth, good symmetry and branching, late flushing, and good needle structure. Both the commercial seedlings and the SE plants in our study originated from this seed source, but with the difference that the standard seedlings were bare-root plants, while the somatic plants were grown in containers. Grossnickle and El-Kassaby [30] found no general rules about bare-root versus containerized seedling growth and establishment, but there seems to be a tendency for large-sized stock types to have a better chance for successful stand establishment. In a Nordmann fir Christmas tree study across three sites and three consecutive years of planting, 4-year-old (large) seedlings were approximately 23 cm higher after 7 years compared to 3-year-old (smaller) seedlings grown from bare roots [31]. In another study of bare-root plants of Nordmann fir across 10 sites, differences in nursery height were traced back 7 years after planting [7]. It is likely that some of the differences in growth between SE plants and standard seedlings can be attributed to differences in initial height. In a review article, Egertsdotter et al. [32] evaluated the field performance of SE plants and found that SE plants generally develop in a similar manner morphologically and physiologically to zygotic plants.

Provenance studies of *Abies* spp. have shown significant differences in growth and the potential to be used as Christmas trees [33,34], as well as in resistance to pathogenic attack [35]. Therefore, identifying good provenances is fundamental to improving product quality. In our study, significant differences were identified among the three geographical clusters of the 27 mother trees used for SE production. In an earlier study of short-distance provenance variation in the Ambrolauri region, height above sea level did not cause any

difference among provenances across three years of seed harvest and planting, evaluated after 7 years [31]. Of course, our study only comprised 11 trees from SW and 8 trees from NE and NW, which were used for seed collection in this study. Therefore, there might be a small sample size effect, although the SE technique amplified the number of copies for seeds from each family, which enhanced the analysis. These results suggest that renewed attention should be given to differences in Christmas tree performance among close-range provenances, but especially to the large differences between families potentially useful in breeding and cloning.

Grafted seed orchards have been used as a proxy to study the clones of Nordmann fir for pathogen susceptibility. Three orchards showed genetic variation and genotype-by-site interactions in their susceptibility to the pathogen *Neonectria neomacrospora* [8]. The estimated H^2 for susceptibility ranged from 0.38 to 0.47, and a significant clone ranking change was shown, indicating that Nordmann fir clones perform differently in different growing environments. The present study is the first to evaluate clonal genetic values with regard to growth, Christmas tree quality, and disorder traits. The results showed pronounced clonal variation and moderate to high broad-sense heritability for growth traits, and quality and disorder traits had low to moderate H^2, from 0.07 to 0.24 and from 0.09 to 0.27, respectively. In our previous progeny test in Nordmann fir [5], narrow-sense heritability (h^2) was estimated for height as 0.46 and for Christmas tree quality as 0.08, which are quite similar to our estimate, even though these estimates were achieved from other provenance materials (Danish sources of Borshomi origin). In our study, the number of branches had low genetic control with a H^2 of 0.10, which is quite different from previous results, which normally show that this trait is under moderate genetic control with a h^2 around 0.2 [5,7]. A similar result was observed for post-harvest needle retention; our H^2 estimates from SE clones were much lower than those of previous results.

Nielsen et al. [16] documented the high impact of previous steps in the SE plant production process as a cause of variation among individuals within the same clone at the nursery stage. The present SE plants were some of the first cloned individuals of Nordmann fir. Limited attention has been given to the production process in order to capture the effect of embryo harvest time (at that time, normal embryo harvest occurs 2–3 times across weeks) and the sorting of embryo quality (only discarding very poor embryos), and none at all to sorting the surviving SE seedlings before planting. Sorting and early selection are ways to obtain more homogeneous plant material [36,37]. Furthermore, some clones had difficulties to varying degrees during SE plant development [38], and some of the clones with few individuals were typically representatives of those genotypes. During SE plant production, most clones were started on the same amount of cell mass and multiplied using the same number of Petri dishes, etc. (unpublished lab notes). These aspects might cause lower broad-sense heritability than is achievable based on today's improved protocols.

Significant changes in ranking for both families and clones suggest that some of the SE clones perform differently in different environments. Our previous study of height across ten sites for half-sib families did not show a G × E interaction for height across well-managed Christmas tree production sites comparable to the sites in this study [7]. Trees in Ry were initially under-planted in an older Christmas tree stand—where the older trees were harvested during the following 2–3 years. This procedure potentially impacts growth and has environmental influences due to competition from larger trees, but some SE plants might also benefit from creating a more protected environment in the first years of establishment. The effect of under-planting potentially slowed the growth in Ry and made trees in this site grow slower than trees established at Holstebro, which was established one year later. In addition to this effect, trees in Lundbygaard generally grew faster than in the other two sites, which is mainly due to the clay soil type in Lundbygaard.

For quality traits, post-harvest needle retention had a significant difference between sites, and trees tended to have more post-harvest needle loss at the southern sites than at the northern site. This could be due to temperature differences, although Denmark is a small country where differences between the frost-free period and average temperatures

are pronounced [39]. This is in concordance with earlier findings [7], where trees grown in colder areas tend to have better needle retention.

As for disorder traits, bare shoulders were much lower at site Ry than in the other two sites. This disorder is poorly understood, but it is generally growers' experience that excessive nitrogen fertilization and limited magnesium supply can partly explain the differences in the disorder, in addition to pronounced site-to-site variations.

5. Conclusions

This study documented how the well-established and well-growing somatic plants of Nordmann fir develop into Christmas trees at three different sites. Pronounced clonal variation was present for the investigated Christmas tree-related traits, and in general, the traits were under low to moderate broad-sense heritability. Variation among families was substantial for all measured traits. The results highlight the importance of superior parent material for cloning, i.e., selection of the best families within the best provenances. Superior clones, compared to the unbred seed source, were identified by combining several superior traits. Genotype-by-site interactions were present for three important traits, indicating the importance of an adequate growing site and of identifying stable performing clones across environments.

Supplementary Materials: The exact geographical coordinates of the 27 mother trees sampling locations including latitude, altitude, and elevation can be downloaded at: https://www.mdpi.com/article/10.3390/f14020279/s1, Table S1: Geographical coordinates of the 27 mother trees sampling locations in the Ambrolauri region in Georgia, Caucasus.

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Data Availability Statement: The datasets generated and/or analyzed in the current study are available at the University of Copenhagen Data Bank.

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References

1. Larson, R.B. Christmas Tree Marketing: Product, Price, Promotion, and Place Tactics. *J. For.* **2004**, *102*, 40–45. [CrossRef]
2. Nielsen, U.B.; Hansen, J.K.; Kromann, H.K. Impact of site and provenance on economic return in Nordmann fir Christmas tree production. *Scand. J. For. Res.* **2011**, *26*, 74–89. [CrossRef]
3. Nzokou, P.; Leefers, L.A. *Costs and Returns in Michigan Christmas Tree Production, 2006*; Michigan State University Extension, MSUE Extension bulletin E-2999: East Lansing, MI, USA, 2007.
4. Nielsen, U.B.; Chastagner, G.A. Variation in Postharvest Quality among Nordmann Fir Provenances. *HortScience* **2005**, *40*, 553–557. [CrossRef]
5. Xu, J.; Nielsen, U.B.; Isik, F.; Jensen, M.; Hansen, O.K. Genetic variation and inheritance of susceptibility to Neonectria neomacrospora and Christmas tree traits in a progeny test of Nordmann fir. *Ann. For. Sci.* **2021**, *78*, 22. [CrossRef]
6. Nielsen, U.B. Breeding noble fir (*Abies procera* Rehder) and Nordmann fir (*Abies nordmanniana* (Stev.) Spach) for Christmas trees and greenery in Denmark. In *The Nordic Group for Tree Breeding*; Forestry Commission: Edinburgh, Scotland, 1994.
7. Nielsen, U.B.; Xu, J.; Hansen, O.K. Genetics in and opportunities for improvement of Nordmann fir (*Abies nordmanniana* (Steven) Spach) Christmas tree production. *Tree Genet. Genomes* **2020**, *16*, 66. [CrossRef]

8. Xu, J.; Hansen, O.K.; Thomsen, I.M.; Nielsen, U.B. Genetic variation and genotype by environment interaction in the susceptibility of Abies nordmanniana (Steven) Spach to the fungus Neonectria neomacrospora (Booth & Samuels) Mantiri & Samuels. *Ann. For. Sci.* **2018**, *75*, 17. [CrossRef]
9. Chalupa, V. Somatic embryogenesis and plantlet regeneration from cultured immature and mature embryos of *Picea abies* (L.). Karst. *Commun. Inst. For. Cech.* **1985**, *14*, 57–63.
10. Hakman, I.; Fowke, L.C.; Von Arnold, S.; Eriksson, T. The development of somatic embryos in tissue cultures initiated from immature embryos of Picea abies (*Norway spruce*). *Plant Sci.* **1985**, *38*, 53–59. [CrossRef]
11. Nagmani, R.; Bonga, J. Embryogenesis in subcultured callus of Larix decidua. *Can. J. For. Res.* **1985**, *15*, 1088–1091. [CrossRef]
12. Nørgaard, J.; Baldursson, S.; Krogstrup, P. Genotypic differences in the ability of embryogenic Abies nordmanniana cultures to survive cryopreservation. *Silvae Genet.* **1993**, *42*, 93.
13. Nørgaard, J.V. Somatic embryo maturation and plant regeneration in Abies nordmanniana Lk. *Plant Sci.* **1997**, *124*, 211–221. [CrossRef]
14. Find, J.I. Towards industrial production of tree varieties through somatic embryogenesis and other vegetative propagation technologies: Nordmann fir (*Abies nordmanniana* (Steven) Spach)-from research laboratory to production. In *Vegetative Propagation of Forest Trees*; National Institute of Forest Science: Seoul, Republic of Korea, 2016; pp. 528–537.
15. Find, J.I. Method for Growing Somatic Embryos of Conifers into Trees. US20200236884A1, 30 July 2020.
16. Nielsen, U.B.; Hansen, C.B.; Hansen, U.; Johansen, V.K.; Egertsdotter, U. Accumulated effects of factors determining plant development from somatic embryos of Abies nordmanniana and Abies bornmuelleriana. *Front. Plant Sci.* **2022**, *13*, 989484. [CrossRef] [PubMed]
17. Blödtner-Piske, C. Die erste klone sind da (The first clones have arrived). *Nadel J.* **2017**, *11–12*, 36–37.
18. Wahid, N.; Lamhamedi, M.S.; Beaulieu, J.; Margolis, H.A.; Deblois, J. Genetic parameters and clonal variation in growth and nutritional traits of containerized white spruce somatic seedlings. *Acta Bot. Gall.* **2012**, *159*, 373–384. [CrossRef]
19. Dean, C.A. Genetic Parameters of Somatic Clones of Coastal Douglas-fir at 5 1/2 -Years across Washington and Oregon, USA. *Silvae Genet.* **2008**, *57*, 4–5. [CrossRef]
20. Dias, P.C.; Xavier, A.; Resende, M.D.V.d.; Barbosa, M.H.P.; Biernaski, F.A.; Estopa, R.A. Genetic evaluation of Pinus taeda clones from somatic embryogenesis and their genotype x environment interaction. *Crop Breed. Appl. Biotechnol.* **2018**, *18*, 55–64. [CrossRef]
21. Weng, Y.H.; Park, Y.S.; Krasowski, M.J.; Tosh, K.J.; Adams, G. Partitioning of genetic variance and selection efficiency for alternative vegetative deployment strategies for white spruce in Eastern Canada. *Tree Genet. Genomes* **2008**, *4*, 809–819. [CrossRef]
22. Grossnickle, S.C.; Major, J.E.; Folk, R.S. Interior spruce seedlings compared with emblings produced from somatic embryogenesis. I. Nursery development, fall acclimation, and over-winter storage. *Can. J. For. Res.* **1994**, *24*, 1376–1384. [CrossRef]
23. Nsangou, M.; Greenwood, M. Physiological and morphological differences between somatic, in vitro germinated, and normal seedlings of red spruce (*Picea rubens* Sarg.). *Can. J. For. Res.* **1998**, *28*, 1088–1092. [CrossRef]
24. Benowicz, A.; Grossnickle, S.C.; El-Kassaby, Y.A. Field assessment of Douglas-fir somatic and zygotic seedlings with respect to gas exchange, water relations, and frost hardiness. *Can. J. For. Res.* **2002**, *32*, 1822–1828. [CrossRef]
25. Lamhamedi, M.S.; Chamberland, H.; Bernier, P.Y.; Tremblay, F.M. Clonal variation in morphology, growth, physiology, anatomy and ultrastructure of container-grown white spruce somatic plants. *Tree Physiol.* **2000**, *20*, 869–880. [CrossRef] [PubMed]
26. Christmas Tree Grower Council of Europe CTGCE. Classification of quality standards for Nordmann Christmas trees. Available online: https://www.ctgce.com/downloads/Classification_1996_E_G_F.pdf (accessed on 6 December 2022).
27. Gilmour, A.R.; Gogel, B.J.; Cullis, B.R.; Welham, S.J.; Thompson, R. ASReml User Guide Release 4.2. VSN International Ltd.: Hemel Hempstead, UK, 2021.
28. Fløistad, I.S.; Nyeggen, H.; Skage, J.-O. Testing species of genus Abies for Christmas tree production in Norway. *Scand. J. For. Res.* **2015**, *30*, 653–663. [CrossRef]
29. Larsen, J.B.; Larsen, B.G.; Kromann, H.K. Abies Nordmanniana provenienser til pyntegrønt og juletræer (Provenances of *Abies nordmanniana* for greenery and Christmas tree production). *Forstl. Forsøgsv. Danm.* **1984**, *39*, 365–382.
30. Grossnickle, S.C.; El-Kassaby, Y.A. Bareroot versus container stocktypes: A performance comparison. *New For.* **2016**, *47*, 1–51. [CrossRef]
31. Nielsen, U.B. Nordmannsgran proveniensforsøg-hvad betyder vækstregulering? *Videnblade Pyntegrønt* **2009**, *3*, 1–26.
32. Egertsdotter, U.; Ahmad, I.; Clapham, D. Automation and scale up of somatic embryogenesis for commercial plant production, with emphasis on conifers. *Front. Plant Sci.* **2019**, *10*, 109. [CrossRef]
33. Madsen, S. Provenance trial of Abies nordmanniana and Abies bornmuelleriana for Christmas tree production in North Sealand. *For. Landsc. Res.* **1994**, *1*, 143–166.
34. Hansen, O.K.; Nielsen, U.B.; Edvardsen, Ø.M.; Skulason, B.; Skage, J.-O. Nordic provenance trials with Abies lasiocarpa and Abies lasiocarpa var. arizonica: Three-year results. *Scand. J. For. Res.* **2004**, *19*, 112–126. [CrossRef]
35. Nielsen, U.B.; Xu, J.; Nielsen, K.N.; Talgø, V.; Hansen, O.K.; Thomsen, I.M. Species variation in susceptibility to the fungus Neonectria neomacrospora in the genus Abies. *Scand. J. For. Res.* **2017**, *32*, 421–431. [CrossRef]
36. Högberg, K.-A.; Bozhkov, P.V.; Von Arnold, S. Early selection improves clonal performance and reduces intraclonal variation of Norway spruce plants propagated by somatic embryogenesis. *Tree Physiol.* **2003**, *23*, 211–216. [CrossRef]

37. Högberg, K.; Varis, S. Vegetative Propagation of Norway Spruce: Experiences and Present Situation in Sweden and Finland. In *Vegetative Propagation of Forest Trees*; Park, Y.S., Bonga, J.M., Moon, H.-K., Eds.; National Institute of Forest Science (Nifos): Seoul, Republic of Korea, 2016; pp. 528–550.
38. Lobo, A.; Find, J.I.; Hansen, J.K.; Ræbild, A.; Kjær, E.D. Effect of temperature and osmotic stress during somatic embryogenesis on phenology and physiology of abies nordmanniana emblings. *For. Ecol. Manag.* **2022**, *514*, 120212. [CrossRef]
39. Jensen, M.; Korsgaard, M.; Pedersen, H.L.; Toldam-Andersen, T.B.; Sørensen, A.H.; Kjærgaard, K.B.; Bjerregaard, G.; Poulsen, J. *Frugt og Bær–Gode Sorter til Haven (Fruit and Berries—Good Varieties for the Garden)*; Århus Universitet: Århus, Denmark, 2019.