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Seedling Growth Responses to Nutrient and Water Treatments Among Jack Pine Open-Pollinated Families

Pengxin Lu [1] and Francis C. Yeh [2,*]

[1] Ontario Forest Research Institute, Ministry of Natural Resources, Sault Ste. Marie, ON P6A 2E5, Canada; pengxin.lu@ontario.ca
[2] Department of Renewable Resources, University of Alberta, Edmonton, AB T6G 2H1, Canada
* Correspondence: fyeh@ualberta.ca

Abstract: Our study, conducted in a controlled greenhouse environment over a single growing season, evaluated the growth of seedlings from 25 open-pollinated families of jack pine (*Pinus banksiana* Lamb.) under two nutrient levels (20 ppm and 200 ppm) and three water regimes (twice a week, once a week, and once every two weeks). We assessed the effects of seed weight, family, nutrient availability, and water treatments on several growth parameters, including height, root collar diameter, shoot dry biomass, root dry biomass, total dry biomass, growing period length, and shoot-to-root ratio at harvest. We found that seed weight significantly influenced all growth traits, maintaining its effect throughout the growth season, although its impact diminished over time. Jack pine families were more responsive to nutrient treatments than to variation in water availability. Genetic variation was significant for all traits except the shoot-to-root ratio, highlighting the intricate role of genetic makeup in shaping growth responses. The substantial impact of nutrient and water treatments and relatively low heritability estimates suggest that pre-conditioning seedlings through nursery management can optimize shoot-to-root ratios. The minimal family-by-treatment interaction and the consistent performance of families across treatments suggest the potential for selecting high-efficiency genotypes with enhanced nutrient use efficiency and drought tolerance.

Keywords: *Pinus banksiana* Lamb.; nutrients; water; seed weight; genetic variation

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1. Introduction

Seedling survival and growth in newly regenerated forests are frequently hindered by prolonged soil drought and low nutrient availability, leading to significant limitations in forest establishment [1,2]. Reduced growth rates not only compromise the competitive ability of seedlings against surrounding herbaceous vegetation for essential resources such as light, water, and nutrients, but also contribute to increased seedling mortality rates [3,4]. While various silvicultural treatments, including thorough site preparation [5] and the application of herbicides [6], have been shown to enhance seedling growth post-regeneration, a more sustainable and cost-effective approach may involve selecting and utilizing seedlings that exhibit superior drought tolerance and nutrient use efficiency [7,8]. This strategy addresses immediate growth challenges and contributes to the long-term resilience of forest ecosystems under changing climatic conditions.

Genetic variation in nutrient use efficiency (NUE) has been extensively studied in tree species, including loblolly pine (*Pinus taeda*) and poplar (*Populus deltoides*). Studies have highlighted that under nutrient-deficient conditions, a strong correlation exists between family-mean seedling size and NUE [8–10]. This correlation suggests that selecting fast-growing genotypes may also indirectly select individuals with higher nutrient use efficiency, offering an effective strategy for improving growth under limited nutrient availability. The same principle extends to drought tolerance, as families exhibiting superior growth under water stress also demonstrate enhanced physiological traits like osmotic adjustment, efficient stomatal control, and higher net photosynthesis [1,11–13]. Additionally, families with solid nursery performance under nitrogen or water-limiting conditions tend to perform well in resource-poor sites [14,15]. This relationship between early-stage resilience and long-term success highlights the importance of selecting genotypes with adaptive traits for nutrient and drought stresses. Current research emphasizes that selecting these traits is especially critical under changing climate scenarios, where increased frequency of droughts and nutrient limitations may pose significant challenges to forest productivity [16,17].

Jack pine (*Pinus banksiana* Lamb.) is a widely distributed species in the boreal forests of North America, extending from the eastern to central regions of Canada and parts of the northern U.S. Its ability to grow on sandy, nutrient-poor soils makes it ecologically significant, but these same conditions present challenges, as drought and low nutrient availability are major limiting factors for growth [18,19]. Understanding the extent of genetic variations in water and nutrient use efficiency within jack pine populations is crucial for developing adaptive breeding strategies to enhance resilience under resource-limited environments [20,21].

This study explores the seedling growth response of 25 open-pollinated jack pine families under different nutrient and water treatments in a controlled greenhouse environment. The objective is to quantify the genetic variability of growth performance and assess the interaction between genotype and environmental factors, particularly for nutrient and water availability. Identifying genotypes with superior drought tolerance and nutrient use efficiency will provide valuable insights for improving jack pine growth under field conditions prone to climate stress and nutrient limitations, as shown for white spruce (*Picea glauca*) [22]. By evaluating genetic responses to these environmental factors, the study aims to inform breeding programs for jack pines, promoting the selection of families that exhibit improved growth and resource use efficiency. These findings will contribute to developing more sustainable and climate-resilient forestry practices, especially given the increasing frequency of drought and changing nutrient dynamics in boreal ecosystems [23].

2. Material and Methods

2.1. Seedling Culture

Seedlings from 25 open-pollinated families of jack pine (*Pinus banksiana* Lamb.) were cultivated in a greenhouse under natural photoperiod conditions and a controlled temperature range of 20–25 °C. Before sowing, the mean seed weight for each family was determined by taking three independent samples of 100 seeds per family. Three seeds from each family were directly sown into individual container pots (2.5 × 2.0 × 9.0 inches, approximately 703 cm^3 in volume) filled with a uniform mixture of peat moss, vermiculite, and sterile sand in a 1:1:1 ratio by volume. Upon germination, seedlings were randomly thinned to one plant per pot to ensure uniform growth, and fungicide was applied following manufacturer guidelines to prevent damping-off disease. Fertilization commenced two weeks after germination, using a complete, water-soluble 20-20-20 fertilizer at a concentration of 200 ppm, applied every two weeks alongside regular watering. Each fertilization was preceded by flushing the pots with tap water to prevent salt accumulation. Fertilizer was applied generously to maintain consistent nutrient concentrations within the growing medium. Additionally, the pH of the irrigation water was adjusted to 5.8 using sulfuric acid to optimize nutrient availability.

Nutrient and water treatments were initiated when the seedlings reached eight weeks of age. The experiment followed 10 × 3 × 2 × 25 randomized complete blocks, with a split-plot design containing ten blocks to ensure robust replication. Each block contained six main plots, where the three water treatments and two fertilizer treatments were randomly assigned. Within each main plot, one seedling from each of the 25 open-pollinated jack pine families was randomly allocated to a subplot to minimize experimental bias.

The three water treatments were designed to simulate varying drought conditions, with watering frequencies of twice a week (W3), once a week (W2), and once every two weeks (W1). The two nutrient treatments represented contrasting nutrient availability conditions: 200 ppm (N2) of a complete soluble fertilizer (20-20-20 with 1.7 ppm iron chelate) to simulate sufficient nutrient availability, and 20 ppm (N1) to simulate nutrient deficiency. Nutrient solutions were applied every two weeks to ensure a consistent experimental regime. This design allowed for the evaluation of the interactive effects of water availability, nutrient levels, and genetic variation across the 25 families on seedling performance.

To enhance seedling diameter and biomass accumulation, high-pressure sodium vapor lamps provided supplemental light with photosynthetically active radiation (PAR) intensity of 111 μmol m^{-2} s^{-1}. The photoperiod was extended to 17 h daily, resulting in a promotion of optimal growth conditions. By this stage, 99% of the seedlings had developed apical terminal buds, indicating the transition to a new growth phase. The lighting conditions and the applied water and nutrient treatments were maintained consistently for 13 weeks until the experiment's conclusion.

2.2. Measurement and Statistical Analysis

Seedling height and root collar diameter were measured both before and after the application of treatments. Final seedling height (H) was defined as the distance from the soil surface to the apex of the terminal bud, while initial height (H_0) was measured from the soil surface to the tip of the uppermost leaves. Weekly observations were conducted to monitor the formation of terminal buds and assess variation among families in response to the progressively shortening natural photoperiod. The presence of a terminal bud was identified by both the cessation of apical growth and the visible emergence of a terminal bud. Each seedling's growing period (LGP) length was calculated as the time elapsed between 1 June 1993, and the date of terminal bud formation.

After the experiment, seedlings were individually harvested, and their tissues were partitioned into shoot biomass (SB), root biomass (RB), and total biomass (TB). All tissue samples were dried at 80 °C for 48 h to determine dry biomass weights. Analysis of variance (ANOVA) was performed to evaluate the effects of nutrient treatments, water regimes, and family on seedling growth. A mixed linear model was employed to account for both fixed and random effects, using the GLM procedure in SAS [24] to analyze all measured traits as follows:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \lambda_k + \alpha\beta_{ij} + \alpha\lambda_{ik} + \beta\lambda_{jk} + \alpha\beta\lambda_{ijk} + \delta_l + \beta\delta_{jl} + \lambda\delta_{kl} + \beta\lambda\delta_{jkl} + \varepsilon_{ijkl} \quad (1)$$

where Y_{ijkl} is the observation of the lth family under the treatment of jth nutrient level and kth water level in the ith block; μ is the overall experimental mean; α_i is the block effect (random); β_j is the nutrient effect (fixed); λ_k is the water effect (fixed); δ_l is the family effect (random); $\alpha\beta_{ij}$ is the block by nutrient interaction; $\alpha\lambda_{ik}$ is the block by water interaction; $\beta\lambda_{jk}$ is the nutrient by water interaction; $\alpha\beta\lambda_{ijk}$ is the block by nutrient by water interaction; $\beta\delta_{kl}$ is the nutrient by family interaction; $\lambda\delta_{kl}$ is the water by family interaction; $\beta\lambda\delta_{jkl}$ is the nutrient by water and by family interaction; and ε_{ijkl} is the residual error.

To assess the effects of nutrient and water treatments, initial seedling height (H_0), measured before treatment application, was included as a covariate to control for pre-existing differences in seedling size. Due to unbalanced data, adjusted (Type III) mean squares were used for all F-tests to ensure accurate statistical inference.

The influence of seed weight (Sdwt) on seedling growth was evaluated through a simple regression model at the family-mean level, applied across each nutrient and water treatment and their combinations. To determine whether the regression parameters were consistent across different treatment levels, the homogeneity of regression slopes between pairs of treatments was tested using the following model:

$$Y_{ij} = b_0 + b_{0i} + b_1 \times Sdwt + b_{1i} \times Sdwt + \varepsilon_{ij} \quad (2)$$

where Y_{ij} is the jth family mean value in seedling growth trait in the ith treatment (or treatment combination); b_0 and b_1 are the intercept and slope, respectively, in the simple regression only using data from treatment level 1; b_{0i} and b_{1i} are additional intercept and slope created by the addition of data from treatment 2 into the model; and ε_{ij} is the error term of regression. A significant b_{0i} indicates a statistically significant difference in the interception of the regression model, suggesting that the effect of seed weight differs between the two treatment levels at the point of interception. Similarly, a significant b_{1i} reflects a difference in the slope of the regression, implying that the influence of seed weight on seedling growth varies across the two treatments. Conversely, non-significant b_{0i} and b_{1i} indicate that the two treatment levels exhibit comparable patterns of seed weight effects, with no meaningful differences in either intercept or slope. Seed weight adjustments were performed following the method outlined by St. Clair and Adams [25].

In addition to the combined analysis, genetic differences among families were evaluated separately for each nutrient and water treatment level and their combinations in cases where treatment effects were statistically significant. Genetic variance components were estimated based on the expected mean squares derived from the corresponding ANOVA models. Heritability was calculated assuming that open-pollinated seeds account for one-quarter of the additive genetic variance, following the method described by Yeh and Rasmussen [26]. The magnitude of family-by-treatment interactions was assessed using Type B genetic correlation [27] and Spearman's rank correlation to determine the stability of family performance across treatments.

3. Results

3.1. Seed Weight Effect

The effect of seed weight was statistically significant for all measured seedling traits, except the length of the growing period (LGP) and the shoot-to-root ratio (S/R) (Table 1).

Table 1. Pearson correlation coefficients and significant levels (below) between family mean seed weight and first-year seedling growth measurements in jack pine (*Pinus banksiana* Lamb.) (levels of statistical significance are in parentheses).

	H_0	H	D	SB	RB	TB	LGP	S/R
N1	0.54 (0.00)	0.44 (0.03)	0.53 (0.01)	0.40 (0.05)	0.50 (0.01)	0.45 (0.02)	0.04 (0.86)	−0.03 (0.89)
N2	0.65 (0.00)	0.54 (0.01)	0.58 (0.00)	0.56 (0.00)	0.57 (0.00)	0.57 (0.00)	0.05 (0.79)	−0.10 (0.63)
N1&N2	0.70 (0.00)	0.51 (0.01)	0.61 (0.00)	0.53 (0.01)	0.58 (0.00)	0.55 (0.00)	0.05 (0.81)	−0.08 (0.72)

Note: H_0 is the seedling height measured before treatment; H, D, SB, RB, TB, LGP, and S/R are, respectively, seedling height, root collar diameter, shoot dry biomass, root dry biomass, total dry biomass, length of growing period, and shoot-to-root ratio measurements at harvest; N1 and N2 are nutrient levels of 20 ppm and 200 ppm, respectively; and N1&N2 is N1 and N2 combined.

The influence of seed weight was more pronounced under high nutrient conditions than under low nutrient availability, as indicated by steeper regression slopes and higher levels of statistical significance. In contrast, no significant differences were observed in the effect of seed weight across the three water treatment levels, suggesting that water availability did not substantially modify the relationship between seed weight and seedling growth.

3.2. Treatment Effects

Regardless of family, seedlings grown under high nutrient conditions (200 ppm) exhibited substantially greater size measurements compared to those grown under low nutrient conditions (20 ppm), with increases of 31% in height (H), 39% in root collar diameter (D), and 119% in total biomass (TB). Highly significant differences ($p < 0.001$) were detected between the two nutrient levels across multiple traits, including H, D, shoot biomass (SB), root biomass (RB), TB, and the shoot-to-root ratio (Table 2).

Table 2. Effect of nutrient (N) treatment, water (W) treatment and their interaction (N × W) on the growth of first-year jack pine (*Pinus banksiana* Lamb.) seedlings as shown by *F* values and their significant levels (in parentheses).

	H	D	SB	RB	TB	LGP	S/R
N	103.19 (0.00)	124.19 (0.00)	92.84 (0.00)	16.89 (0.00)	71.65 (0.00)	44.18 (0.00)	166.04 (0.00)
W	1.33 (0.32)	15.55 (0.00)	11.05 (0.00)	6.03 (0.01)	9.36 (0.00)	3.73 (0.06)	15.65 (0.00)
N × W	7.65 (0.03)	1.23 (0.31)	0.96 (0.41)	0.36 (0.70)	0.67 (0.53)	5.96 (0.01)	0.19 (0.83)

Note: H, D, SB, RB, TB, LGP, and S/R are, respectively, seedling height, root collar diameter, shoot dry biomass, root dry biomass, total dry biomass, length of growing period, and shoot-to-root ratio measurements at harvest.

Water availability also significantly impacted D, SB, RB, TB, and the shoot-to-root ratio, though it did not affect H (Table 2). Significant growth differences were observed between the W1 treatment (watering every two weeks) and both W2 (once a week) and W3 (twice a week). However, minimal differences were detected between W2 and W3, suggesting that the effect of increased watering frequency plateaued beyond once per week.

Low nutrient availability strongly promoted the formation of apical terminal buds, particularly when combined with severe water stress (W1), as shown in Figure 1. On average, the growing period (LGP) length was 3.64 days shorter under low nutrient conditions than high nutrient conditions, indicating that nutrient limitations accelerated the onset of bud dormancy. The interaction between nutrient and water treatments (N × W) was statistically significant only for LGP and final seedling height (H). No significant N × W interaction effects were detected for all other traits, suggesting that nutrient and water treatments independently influenced most growth traits.

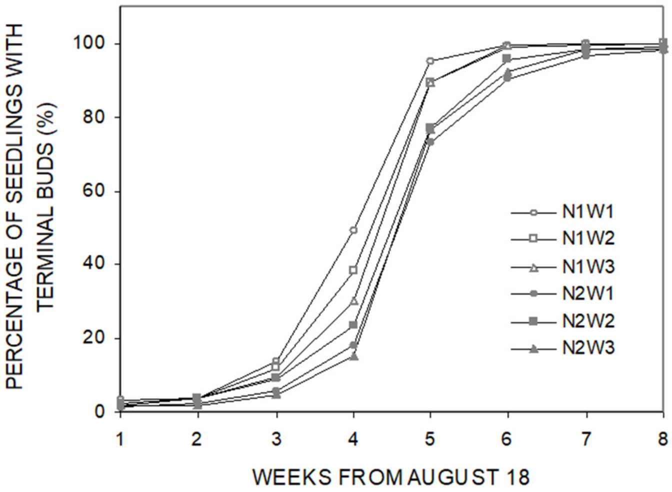

Figure 1. Low nutrient supply and soil moisture stress promoted the terminal bud formation of jack pine seedlings. N1 and N2 are nutrient levels of 20 ppm and 200 ppm, respectively, and W1, W2, and W3 are irrigation frequencies of once every two weeks, once a week and twice a week, respectively.

3.3. Genetic Variation Among Families

The combined analysis showed significant genetic differences among the 25 open-pollinated jack pine families for all seedling traits except for the shoot-to-root ratio. Across treatments, the best-performing family outgrew the poorest-performing family by 65.46% in height (H), 43.40% in root collar diameter (D), and 172.60% in total biomass (TB). Family variance components accounted for 5.08%–20.34% of the total phenotypic variation in seedling size and dry biomass, with the most significant contribution observed for seedling height and the smallest for total biomass (Table 3). Genetic variation was more pronounced under high nutrient conditions, as indicated by higher estimates of the genetic variation coefficient (g.v.c.).

Table 3. Variance component estimates and percents accounting for total phenotypic variance (in parentheses) for family (F), family × nutrient (FN), family × water (FW), and family × nutrient × water interactions (FNW) for seven seedling traits. Family and individual heritability estimates were also shown with standard error estimates in parentheses.

	F	**FN**	**FW**	**FNW**	**ERROR**	**h_f^2**	**h_i^2**
H	37.1380 ** (20.34)	3.0665 (1.31)	0 (0)	1.7717 (0.97)	140.5817 (77.01)	0.90 (0.04)	0.81 (0.06)
D	0.0174 ** (9.36)	0.0050 (2.70)	0 (0)	0.0083 (4.49)	0.1551 (83.47)	0.76 (0.12)	0.37 (0.09)
SB	0.0317 ** (8.27)	0.0268 ** (6.99)	0.0035 (0.90)	0.0076 (0.20)	0.3208 (83.65)	0.61 (0.15)	0.33 (0.11)
RB	0.0088 ** (7.81)	0.0017 (1.55)	(0) (0)	0.0007 (0.65)	0.0990 (88.43)	0.77 (0.14)	0.32 (0.08)
TB	0.0417 ** (5.08)	0.0353 * (4.30)	(0) (0)	0.0063 (0.76)	0.7381 (89.86)	0.68 (0.15)	0.20 (0.10)
S/R	0.0062 (0.71)	0.0151 (1.74)	0.0003 (0.003)	(0) (0)	0.8465 (97.51)	0.21 (0.34)	0.03 (0.05)

H, D, SB, RB, TB, LGP, and S/R are, respectively, seedling height, root collar diameter, shoot dry biomass, root dry biomass, total dry biomass, length of growing period, and shoot-to-root ratio measurements at harvest. * and ** indicate statistical significance at the $p < 0.05$ and $p < 0.01$ levels.

The g.v.c. values were calculated as the ratio of the square root of the family variance component to the mean of the corresponding growth measurement at each treatment level (Table 4). Although absolute family differences in growth were minor under low nutrient conditions, they remained statistically significant. This is attributed to the narrower variation within families, emphasizing the relative differences among families even under resource-limited conditions.

Table 4. Mean growth and genetic variation coefficient (g.v.c.) of jack pine (*Pinus banksiana* L.) seedlings under two nutrient (N = 20 and 200 ppm) and three water (W = watering twice a week, once a week, and once every two weeks) treatments.

		N1	**N2**	**W1**	**W2**	**W3**
H	mean	42.35	54.21	48.12	48.77	47.95
	g.v.c.	0.125	0.133	0.120	0.121	0.138
D	mean	1.26	1.72	1.36	1.55	1.56
	g.v.c.	0.081	0.107	0.071	0.063	0.093
SB	mean	0.87	1.81	1.14	1.42	1.46
	g.v.c.	0.220	0.246	0.167	0.177	0.251
RB	mean	0.39	0.52	0.40	0.49	0.49
	g.v.c.	0.177	0.241	0.117	0.182	0.239
TB	mean	0.87	1.81	1.14	1.42	1.46
	g.v.c.	0.195	0.242	0.154	0.178	0.251
LGP	mean	96.60	100.31	98.16	98.07	99.14
	g.v.c.	0.022	0.033	0.023	0.027	0.022
S/R	mean	1.34	2.93	1.90	2.13	2.30
	g.v.c.	0.086	0.060	0.066	0.028	0.062

H, D, SB, RB, TB, LGP, and S/R are, respectively, seedling height, root collar diameter, shoot dry biomass, root dry biomass, total dry biomass, length of growing period, and shoot-to-root ratio measurements at harvest. The genetic variation coefficient (g.v.c.) is calculated as the square root of the family variance component divided by the treatment mean value.

The shoot-to-root dry biomass ratio exhibited significant differences among families under low nutrient conditions (N1). Nevertheless, it showed no significant variation at the high nutrient level (N2) or across the three water treatment levels. Under most nutrient- and water-limited conditions, fast-growing families displayed relatively low shoot-to-root ratios but still produced greater absolute root biomass than slow-growing families. A significant positive correlation ($p < 0.05$) was found between seedling height growth and the shoot-to-root ratio under N1 and W1 conditions, while no significant correlations were observed under N2, W2, or W3 conditions.

The family × water treatment interaction was minimal across all measured traits, indicating that family performance was largely stable across water regimes. However, the family × nutrient treatment interaction was statistically significant ($p \leq 0.05$) for several traits, though the ratio of the interaction variance component to the family variance component did not exceed one-third for height (H) or root collar diameter (D). Type B genetic correlations between the two nutrient levels were high for most traits ($r_g > 0.88$ for H, D, SB, RB, TB, and LGP). This suggests that families with superior performance under low nutrient conditions also maintained strong growth at high nutrient levels.

Heritability estimates were generally high across all seedling traits except for the shoot-to-root ratio (Table 3). Genetic and phenotypic variation among key growth traits—height (H), root collar diameter (D), shoot biomass (SB), root biomass (RB), and total biomass (TB)—were consistently large as shown by the g.v.c. estimates, indicating a close association between these traits. However, genetic variation in the shoot-to-root ratio water was largely negligible, primarily due to the high standard error estimates. Similarly, no meaningful genetic variation was detected in the length of the growing period (LGP) (Table 4), suggesting that these traits develop independently.

4. Discussion

4.1. Seed Weight Effect

The significant family mean correlation between seed weight and early seedling growth observed in this study aligns with previous research on jack pines [18] and other temperate conifer [28–30] and tropical rainforest tree species [31]. In our findings, seed weight explained 49% of the variation in seedling height at eight weeks but only 26% at six months, indicating a diminishing influence of seed weight on growth over time. Despite this decline, a positive correlation ($r = 0.31$; $p \leq 0.13$) between family mean seed weight and the 15-year field height suggests a weak but persistent long-term effect of seed weight on tree growth. This result supports prior findings that seed weight contains both maternal effects and additive genetic variation [25,29], influencing early performance and long-term growth outcomes.

The significant effect of seed weight on early jack pine growth suggests the practical benefits of selecting heavier seeds to produce faster-growing seedlings, which, in turn, may lead to higher growth rates after planting [32]. Notably, the non-significant correlations between seed weight and the length of the growing period indicate that seed weight promotes growth rates without extending the growing period. This characteristic is crucial for boreal forest species like jack pine, as it implies that culling based on seed weight would not expose seedlings to early forest damage or late-season environmental stress, which could otherwise occur if growth was delayed into unfavorable periods.

Interestingly, the more substantial seed weight effects under the high nutrient treatment in this study contrast with findings in slash pine, where seed weight had a more pronounced impact under low nitrogen conditions [33]. This disparity may reflect species-specific differences in nutrient use efficiency or growth strategies. However, it could also result from sampling variation, as seedlings at the high nutrient level in this study exhibited slightly stronger seed weight effects even before treatments were applied, suggesting that initial conditions may have amplified these effects during the experimental period.

These results underscore the importance of seed selection based on weight for nursery practices, particularly when optimized for early growth. They also highlight the need for further research into species-specific nutrient interactions to better inform reforestation and afforestation strategies.

4.2. Seedling's Growing Period

Both growth rate and the duration of the active growth period generally influence seedling size accumulation. In this study, nutrient and water availability in the growth medium significantly affected the length of the growth period across all families. A more extended growth period was observed under conditions of adequate nutrient and water supply during the late stages of seedling development, consistent with findings that resource availability extends active growth in conifer species [34,35].

Despite the significant family-level variation in the growth period length, no significant genetic correlation was detected between the length of the growth period and seedling size (Table 5). This result suggests that a more extended growth period does not necessarily translate into superior growth performance in jack pines. While extended growth duration may offer advantages, high growth rates are generally more critical for seedling performance due to their role in resource capture, stress tolerance, and early establishment. This focus on maximizing growth rate, rather than growth duration, aligns with the demands of forestry operations, where seedlings must adapt quickly to varied field conditions and demonstrate long-term productivity [36,37].

Table 5. Genetic (above diagonal) and phenotypic (below diagonal) correlation estimates among seedling traits (standard errors of estimates are in parentheses).

	H	**D**	**SB**	**RB**	**TB**	**LGP**
H		0.93 (0.03)	0.95 (0.09)	0.94 (0.04)	0.95 (0.07)	0.04 (0.78)
D	0.78 (0.01)		0.93 (0.02)	0.98 (0.01)	0.95 (0.01)	0.15 (0.91)
SB	0.79 (0.01)	0.86 (0.01)		0.99 (0.00)	1.00 (0.00)	0.10 (0.93)
RB	0.64 (0.01)	0.75 (0.01)	0.83 (0.03)		1.00 (0.00)	0.00 0.96
TB	0.77 (0.01)	0.86 (0.01)	0.98 (0.00)	0.92 (0.02)		0.07 (0.93)

Note: H, D, SB, RB, TB, and LGP are, respectively, seedling height, root collar diameter, shoot dry biomass, root dry biomass, total dry biomass, and length of growing period.

The absence of a strong genetic link between these two traits has important implications for breeding programs. Breeders can select fast-growing families with shorter growth periods without compromising overall seedling size. This strategy is precious in regions prone to early frost events, as a reduced growth period minimizes the risk of frost damage to late-season growth [38]. These findings emphasize the importance of targeting growth efficiency rather than just extending growth periods when selecting genotypes for reforestation or afforestation in climates with early onset of harsh weather conditions.

4.3. Treatment Effects and Genetic Differences

The highly significant response of jack pine seedlings to nutrient availability highlights the species' sensitivity to soil nutrient conditions and underscores the substantial environmental differences introduced by the nutrient treatments. If a nutrient concentration of 200 ppm is sufficient to meet the physiological demands of first-year jack pine seedlings, as Scarratt [39] reported, the 20 ppm concentration was likely below the minimum threshold required for optimal growth. Seedlings subjected to this low nutrient level displayed symptoms of nitrogen deficiency during the later stages of the experiment, consistent with prior research showing that nutrient-poor conditions can hinder growth and photosynthetic capacity in conifers [35,39].

The variation in growth among open-pollinated families under nutrient-limited conditions likely reflects genetic differences in resource-use efficiency and the ability to utilize limited nutrients effectively. Such family-level variation aligns with findings from other conifer species, which demonstrate that genetic factors can influence the ability to sustain growth under suboptimal environmental conditions [34,40]. These results suggest that, even in nutrient-stressed environments, certain jack pine families possess inherited traits that enable them to outperform others, indicating the potential for selecting genotypes with enhanced nutrient-use efficiency for reforestation programs.

The weaker effect of water treatments on seedling height growth, compared to their more pronounced impact on basal diameter and dry biomass accumulation, likely reflects water balance dynamics throughout the experimental period. In the early stages of the experiment, seedlings exhibited limited shoot and leaf development, resulting in low transpiration rates. Meanwhile, the rapid growth of the root systems allowed seedlings to access deeper water reserves within the soil. Given the relatively deep container pots used in the experiment, the soil retained sufficient moisture to support average seedling growth across treatments, even with the W1 watering regime (watering every two weeks).

As seedlings grew, their water demand increased, particularly during the later stages when shoot size expanded, and needle production intensified. However, any water stress experienced under the W1 and W2 regimes (watering every two weeks and once a week, respectively) did not appear severe enough to significantly limit height growth until the formation of terminal buds in late August. This finding aligns with previous studies suggesting that height growth in conifers tends to remain unaffected by moderate water deficits until the onset of bud dormancy [41].

In contrast, basal diameter and biomass accumulation were more sensitive to water availability, particularly during the later stages of the experiment, when larger seedlings and greater needle production resulted in higher water demand. Under conditions of water limitation, these traits likely became constrained, as water stress during the late growing period has been shown to disproportionately impact radial growth and biomass production in conifers [42,43].

This study highlights the importance of recognizing that all seedlings were cultivated under equal growth spaces, ensuring uniform access to nutrients and water. However, because a seedling's nutrient and water demand increase with size [38], it is reasonable to assume that fast-growing families will likely experience more nutrient and water stress than slower-growing families. Our xylem water potential measurements, which indicated increased water stress among larger seedlings (Table 6), support this hypothesis.

Table 6. Seedling xylem water potential (bar) under different nutrient and water treatment conditions before harvesting.

	W1 (n*)	W3 (n*)	Mean (n*)
N1	10.54 ± 3.66 (25)	8.92 ± 1.05 (25)	9.73 ± 2.79 (50)
N2	18.86 ± 5.17 (23)	9.56 ± 1.90 (25)	14.21 ± 6.04 (48)
Mean	14.37 ± 6.05 (48)	9.22 ± 1.54 (50)	

*n = number of seedlings measured. W1 and W3 represent watering once every two weeks and watering twice a week, respectively. N1 and N2 represent nutrient treatment at 20 ppm and 200 ppm, respectively.

Evaluating seedlings under uniform growth conditions may have underestimated the genetic differences among open-pollinated families, especially in nutrient- and water-limited environments. Competition for resources amplifies stress in natural or field conditions, and differences in the genetic capacity to efficiently utilize limited resources become more pronounced [34,35]. Thus, while controlled environments provide insights into potential growth performance, they may not fully capture the extent of genotypic variability under resource-limited conditions, underscoring the need for further evaluation in more variable field settings.

Substantial genetic variation across all treatment combinations highlights the potential for selecting superior genotypes with enhanced nutrient-use efficiency and tolerance to water stress. Genetic variation in seedling growth was meaningful because open-pollinated families would theoretically exhibit only one-quarter of genetic merits of their mother trees. However, genetic superiority under severe nutrient or water stress conditions alone may not be sufficient to identify an ideal genotype. To achieve optimal growth under favorable conditions, a desirable genotype must also demonstrate a strong positive response to improved nutrient and water availability [34,35].

In this study, families that performed well under nutrient- and water-limited conditions also exhibited superior growth under favorable environmental conditions, indicating high stability across different treatments. This is supported by the high Type B genetic correlations, weak genotype-by-environment (G × E) interactions, and strong Spearman's rank correlations across the six nutrient and water treatment combinations (Table 7). These findings align with previous reports of stable performance in jack pine families [44], suggesting that genetic stability is a crucial characteristic of this species.

Table 7. Spearman family rank correlations among six combinations of nutrient (N1 = 20 ppm, N2 = 200 ppm) and water treatment levels (W1 = watering twice a week, W2 = watering once a week and W3=watering once every two weeks) in seedling height (H) (upper diagonal) and seedling total dry biomass growth (TB) (lower diagonal) with the significant probability levels in parentheses.

	N1W1	**N1W2**	**N1W3**	**N2W1**	**N1W2**	**N2W3**
N1W1		0.86 (0.00)	0.90 (0.00)	0.79 (0.00)	0.82 (0.00)	0.83 (0.00)
N1W2	0.65 (0.00)		0.90 (0.00)	0.72 (0.00)	0.75 (0.00)	0.77 (0.00)
N1W3	0.75 (0.00)	0.73 (0.00)		0.72 (0.00)	0.81 (0.00)	0.78 (0.00)
N2W1	0.51 (0.01)	0.58 (0.00)	0.62 (0.00)		0.58 (0.00)	0.77 (0.00)
N2W2	0.73 (0.00)	0.65 (0.00)	0.68 (0.00)	0.62 (0.00)		0.75 (0.00)
N2W3	0.69 (0.00)	0.66 (0.00)	0.77 (0.00)	0.66 (0.00)	0.68 (0.00)	

Note: N stands for nutrient treatment, and W stands for water treatment. The numbers after N and W are their appropriate treatment levels.

The evidence of stable performance across environmental gradients implies that families with solid growth under nutrient- and water-stressed conditions are also likely to perform well under improved conditions. Therefore, jack pine genotypes with superior growth under resource-limited conditions may represent ideal candidates for reforestation or afforestation, as they are expected to achieve higher growth rates when planted in both marginal and favorable environments.

Under water- and nutrient-stressed conditions, more significant family-level differences and higher heritability estimates for the shoot-to-root ratio were observed, aligning with findings reported by Li et al. (1991c) [45]. A higher allocation of total dry biomass to the root system is often associated with faster growth rates, as Cannell (1978) [14] and Li et al. (1991c) [45] noted. For example, Li et al. (1991c) [45] demonstrated that the regression slope of root dry biomass on total dry biomass, derived using Huxley's equation, was significantly correlated with family mean seedling height in loblolly pine under low nitrogen conditions. Similarly, Cannell (1978) found that the slope of shoot-to-root biomass differed among families and was negatively correlated with 8-year family mean height growth on a moderately well-drained site, suggesting that lower shoot-to-root ratios favor long-term growth.

However, the results of this study suggest that fast-growing jack pine families, although exhibiting greater absolute root biomass, tended to have a lower shoot-to-root ratio in terms of absolute weight under low nutrient conditions and severe water stress conditions. This finding is supported by the significantly positive Pearson correlation between the shoot-to-root ratio and mean family seedling height. This indicates that fast-growing families may allocate relatively more biomass to the shoot than to the root system.

In contrast to previous studies, no significant correlations were detected between the regression slopes from Huxley's equation and mean family growth, suggesting that biomass partitioning patterns may vary among species and environments. Thus, for fast-growing jack pine families, efficient nutrient utilization—rather than nutrient uptake through extensive root growth, as defined by Li et al. [9], may be a more critical factor for maintaining growth under stress conditions. Similar trends have been reported in black spruce (*Picea mariana*), where faster-growing families exhibited lower root-to-shoot ratios under resource limitations [46]. These findings underscore the importance of targeting nutrient-use efficiency in breeding programs to enhance the resilience of jack pine under adverse environmental conditions.

4.4. Implications to Jack Pine Regeneration

Paterson and Maki's regeneration study on jack pines (*Pinus banksiana*) [32] emphasized the importance of maintaining a low shoot-to-root ratio in planting stock to enhance survival during transplanting shock. At the same time, the study highlighted that the initial seedling height plays a critical role in sustaining high growth rates for several years post-transplant, with effects persisting up to six years after planting. Therefore, achieving quality regeneration requires planting stock that balances a reasonable shoot-to-root ratio with genetic superiority in growth rate.

This ideal combination can be effectively achieved through pre-conditioning treatments in nurseries and genetic selection [47]. Given that the shoot-to-root ratio has low heritability and is highly responsive to environmental factors such as nutrient and water conditioning, pre-conditioning seedlings through water stress or nutrient withholding can regulate the shoot-to-root ratio across all seedlings. Such practices ensure that seedlings develop optimized root systems for improved survival while avoiding excessive shoot development, which could compromise field performance under transplant stress [48].

Moreover, the high heritability and stability of jack pine's growth traits, such as height (H) and diameter (D), make selecting families with superior growth rates feasible under unrestricted nursery conditions. These selected seedlings are expected to exhibit consistent performance across favorable and stressed field environments, ensuring adaptability and productivity [49]. Therefore, nursery pre-conditioning and genetic selection are effective strategies for producing planting stock with resilience and growth potential under varying environmental conditions.

5. Conclusions

Open-pollinated jack pine families exhibited considerable and meaningful genetic variation in first-year seedling growth in an environmentally controlled greenhouse experiment with simulated nutrient and water availability levels. Nutrient treatment resulted in a more significant difference in seedling growth than in water stresses applied. Jack pine families, however, displayed consistent performance across nutrient and water treatments, revealing a potentially fast and cheap approach to selecting superior genotypes for tree improvement. Greenhouse experiments using jack pine seedlings under controlled environments minimized experimental noise and increased heritability and selection precision estimates. Future studies are needed to validate greenhouse experimental results with more mature trees growing in the field where they encounter various natural environmental stresses.

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