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Posted Date: 27 May 2025

doi: 10.20944/preprints202505.2060.v1

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Article

Genetic Variation and the Relationships Among Growth, Morphological, and Physiological Traits in *Pterocarpus macrocarpus*: Implications for Early Selection and Conservation

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Abstract: Understanding genetic variation in commercially vital tree species is critical for optimizing breeding and conservation strategies. This study evaluates genetic variation, heritability, and trait correlations in *Pterocarpus macrocarpus*, a high-value hardwood central to Thailand's reforestation efforts. We assessed growth (height, diameter), morphological (biomass dry weight, specific leaf weight), and physiological traits (net photosynthesis [*A*], transpiration rate [*E*], water-use efficiency [WUE]) across 112 open-pollinated families from six natural populations under controlled nursery conditions over 30 weeks. Using a randomized complete block design, variance and covariance analyses were conducted to estimate genetic parameters. Seedling survival reached 95%, confirming favorable conditions for genetic expression. Populations and families within populations exhibited significant divergence in growth and biomass traits, while physiological traits showed pronounced family-level variation (*A*, *E*, WUE) and population effects for WUE only. Residual variance dominated across traits, highlighting substantial within-family variation. Growth and biomass traits demonstrated moderate to high heritability (individual: 0.386–1.000; family: 0.614–0.895), whereas specific leaf weight and shoot-to-root ratio displayed lower individual heritability. Physiological traits exhibited low to moderate heritability (individual: 0.256–0.428; family: 0.473–0.615), with maternal effects (via seed weight) significantly influencing early growth. Height heritability declined over time, contrasting with stable diameter heritability. Strong genetic correlations among growth and biomass traits suggest potential for simultaneous selection gains. Physiological traits, however, exhibit weak or no correlation with growth, highlighting their independent genetic regulation. Population-level variation in growth and WUE may illustrate adaptive responses to seed-source environments. Our findings support nursery-based screening as a cost-effective tool for the early selection of superior families. WUE serves as a promising target for breeding programs focused on drought-prone sites. This study provides foundational insights for advancing *P. macrocarpus* genetic improvement and conservation, emphasizing the integration of physiological traits into breeding and conservation strategies.

Keywords: *Pterocarpus macrocarpus*; growth-morphology-physiology traits; genetic structure; heritabilities; genetic correlations; early selection; conservation

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1. Introduction

Genetic diversity is the cornerstone of forest resilience, enabling tree species to adapt to environmental fluctuations, resist pests and diseases, and maintain ecosystem functionality amidst global change [1,2]. In an era marked by rapid deforestation, habitat fragmentation, and climate instability, deciphering patterns of genetic variation has become a scientific imperative to safeguard biodiversity and optimize the productivity and sustainability of reforestation programs [3,4]. Genetic variation exists hierarchically, partitioned among populations due to divergent selection pressures, founder effects, or genetic drift, and within populations as a result of mutation, recombination, and balancing selection [5–8]. The relative proportion of these components dictates breeding and conservation priorities: high interpopulation divergence necessitates provenance-based strategies to preserve locally adapted gene pools, while pronounced intrapopulation diversity underscores the value of within-population selection to capture superior genotypes [8,9]. However, genetic architecture is not static; it varies across traits, developmental stages, and environmental gradients [10,11]. For example, growth traits may exhibit strong additive genetic control in saplings but become increasingly influenced by environmental heterogeneity as trees mature [12,13]. Such complexity demands a nuanced understanding of trait heritability and plasticity to design breeding and conservation programs that are resilient to climatic uncertainty.

While significant strides have been made in quantifying the genetic control of growth and structural traits (e.g., height, stem form, wood density), physiological traits, such as photosynthetic efficiency, stomatal regulation, and nutrient-use efficiency, remain disproportionately understudied in forestry research [14,15]. This knowledge gap arises from historical biases toward easily measurable field traits, logistical challenges in scaling physiological measurements, and misconceptions that physiological processes are too environmentally labile to serve as reliable selection criteria [16,17]. Yet, physiological traits are the mechanistic bridge between genotype and phenotype, directly mediating a tree's ability to optimize carbon gain, mitigate water stress, and acclimate to abiotic extremes [18]. Recent advances in phenotyping technologies and growing recognition of climate-driven selection pressures have reignited interest in physiological traits as early indicators of adaptive potential [19,20]. For instance, water-use efficiency (WUE), which reflects the trade-off between carbon assimilation and transpiration, has emerged as a critical trait for breeding drought-resilient genotypes in water-limited ecosystems [11,21]. Nevertheless, integrating physiological traits into operational breeding programs remains in its early stages, hindered by limited empirical data on their heritability and genetic correlations with productivity traits.

Pterocarpus macrocarpus Kurz (Fabaceae), a nitrogen-fixing hardwood endemic to the seasonal tropical forests of mainland Southeast Asia, epitomizes a species for which bridging this knowledge gap is both ecologically urgent and socioeconomically strategic. Renowned for its dense, termite-resistant timber and ornamental value, *P. macrocarpus* is a keystone species in Thailand's reforestation initiatives, where it is extensively planted to restore degraded watersheds, combat soil erosion, and provide livelihood opportunities through community forestry [22–25]. Its ecological versatility, tolerating nutrient-poor soils, seasonal drought, and fire, makes it a candidate for climate-smart reforestation. However, despite its prominence, the species lacks structured breeding programs, and existing genetic studies are limited to population diversity assessments using neutral markers. Isozyme analyses reveal striking genetic differentiation among Thai populations, with a clear east–west cline likely shaped by historical biogeographic barriers and environmental gradients [26]. Yet, neutral markers provide little insight into adaptive genetic variation or the heritability of traits critical to reforestation success. This disconnect underscores the need for trait-based studies to unlock the species' full potential in restoration ecology.

Nursery trials offer a pragmatic yet underutilized platform for advancing such research. By providing controlled growing conditions, nurseries minimize environmental noise, allowing genetic differences among families and populations to manifest more clearly than in heterogeneous field environments [27]. This setting is particularly advantageous for assessing physiological traits like photosynthetic rates and WUE, which are sensitive to microenvironmental fluctuations and difficult to measure at scale in natural stands [28,29]. Early-life trait expression in nurseries also enables rapid screening, circumventing the decades-long wait for maturity in slow-growing hardwoods. While age-age correlations between nursery and field performance can weaken due to shifting selection pressures over ontogeny [30–32], nursery data remain invaluable for eliminating poorly adapted families before costly, long-term field trials [33,34]. Moreover, nursery-based heritability estimates provide baseline parameters for predicting genetic gain and refining breeding strategies, a critical step for species like *P. macrocarpus*, where no prior breeding infrastructure exists.

In this study, we bridge these scientific and practical gaps by conducting a comprehensive genetic evaluation of *P. macrocarpus* under nursery conditions. Using open-pollinated progeny from 112 families across six natural populations in Thailand, we (1) quantify genetic variation in growth (height, diameter), morphological (biomass allocation, specific leaf weight), and physiological traits (net photosynthesis, transpiration, WUE); (2) estimate trait heritabilities and maternal effects; and (3) elucidate genetic correlations among traits to identify potential selection trade-offs or synergies. Integrating physiological traits into a traditional growth-morphology framework advances a holistic understanding of *P. macrocarpus*' genetic architecture, providing actionable insights for breeding programs to enhance productivity and climate resilience in Southeast Asia's threatened tropical forests.

2. Materials and Methods

2.1. Plant Material and Experimental Design

2.1.1. Seed Sources and Genetic Background

Open-pollinated seeds of *Pterocarpus macrocarpus* were collected from 112 maternal families across six natural populations in Thailand (Table 1), representing a subset of 287 families from 11 populations previously analyzed for isozyme variation [26]. These populations were selected to capture geographic and climatic diversity, spanning distinct forest types (deciduous dipterocarp, mixed deciduous, and dry evergreen) and varying environmental gradients (Table 1). Inbreeding coefficients (F) and multilocus outcrossing rates (t_m), were derived from prior parentage analyses [35] to describe the mating system (Table 1).

2.1.2. Seed Preparation and Germination Protocol

Eighty seeds per family were individually weighed (±0.001 g) to assess seed mass variability. To overcome physical dormancy, seeds were scarified using medium-grit sandpaper (P80 grade) to abrade the seed coat. Seeds were sown in 24-cell multipot trays (cell dimensions: 5.1 cm diameter × 10.2 cm depth; 190 ml volume) filled with a sterile substrate composed of coconut husk fibre, coarse sand, and compost (2:1:1 v/v), adjusted to a pH of 7.5 with dolomitic limestone. Trays were arranged in a randomized family-block layout to minimize positional bias. Sowing occurred in late May to align with Thailand's rainy season, and germination was monitored daily. Germination (radicle emergence ≥2 mm) concluded within 10 days, with final germination rates calculated at 14 days post-sowing.

2.1.3. Nursery Management and Transplanting

Seedlings were maintained in a shaded nursery under controlled conditions of 27°C (±2°C), 80% relative humidity (RH), and 680 μmol m^{-2} s^{-1} of photosynthetic active radiation (PAR). At three weeks post-sowing, each seedling received 0.5 g of controlled-release fertilizer (Osmocote 13-13-13, N-P-K; ICL Specialty Fertilizers) to promote early growth. After 12 weeks, 16 robust seedlings per family (1,792 total) were selected based on uniformity in height (±10% deviation) and the absence of visible stress symptoms (e.g., chlorosis, wilting). Seedlings were transplanted into 3-L terracotta pots (17.8 cm diameter × 20.3 cm depth) filled with the same substrate mixture and arranged in a randomized complete block design (RCBD) with 16 replications (single-tree plots). Pots were spaced 20 cm apart to prevent canopy overlap and ensure uniform light exposure.

2.1.4. Fertilization and Environmental Controls

Irrigation was applied every 48 hours via drip lines to maintain substrate moisture at 60–70% of field capacity. Additional fertilizer applications (10 g Osmocote 13-13-13 per seedling) occurred at weeks 12 and 21 to sustain nutrient availability. Environmental conditions were rigorously monitored using HOBO data loggers (Onset Computer Corporation), which recorded mean daily temperatures of 27 °C (range: 24–30 °C), relative humidity (RH) of 80% (±5%), and photosynthetically active radiation (PAR) levels of 680 μmol m^{-2} s^{-1} (±50 μmol) during the 30-week experiment.

2.2. Trait Assessments

2.2.1. Growth and Morphological Traits

Height and Diameter: Seedling height (cm) was measured weekly from the soil surface to the apical meristem using a rigid ruler (±1 mm precision), starting at week 3 post-sowing. Root collar diameter (mm) was measured at the soil interface with digital calipers (Mitutoyo Corp.; ±0.01 mm precision) beginning at week 12. Data were collected at 3-week intervals, resulting in 10 height and seven diameter measurements per seedling.

Biomass Partitioning: At harvest (30 weeks), seedlings were carefully uprooted, washed to remove the substrate, and partitioned into leaves, stems, taproots (>2 mm diameter), and fibrous roots (<2 mm diameter). Tissues were oven-dried at 80°C for 48 hours until a constant mass was achieved, and dry weights (g) were recorded using an analytical balance (Mettler Toledo; ±0.001 g precision). Specific leaf weight (SLWT; g cm^{-2}) was calculated as leaf dry mass divided by total leaf area. Leaf area was quantified using a portable laser area meter (CI-202, CID Bio-Science) on four fully expanded leaves per seedling, selected from the mid-canopy to minimize positional variability.

2.2.2. Physiological Traits

Gas exchange parameters were measured at week 27 using an infrared gas analyzer (IRGA; CI-301PS, CID Bio-Science) with a 1-L leaf chamber. Two mature, sun-acclimated leaves per seedling were selected from the upper third of the canopy to represent photosynthetic capacity under light-saturated conditions. Measurements were conducted between 08:30 and 15:30 under ambient sunlight (PAR: 1,026–2,090 μmol m^{-2} s^{-1}) and temperatures ranging from 25 to 34°C. The net photosynthetic rate (A; μmol CO_2 m^{-2} s^{-1}) and transpiration rate (E; mmol H_2O m^{-2} s^{-1}) were recorded over 30-second intervals following a 90-second chamber equilibration. Instantaneous water-use efficiency (WUE; μmol CO_2 $mmol^{-1}$ H_2O) was calculated as A/E [36]. The measurement order was randomized across replications to minimize diurnal variability, and chamber conditions were calibrated hourly using a certified CO_2 reference gas (400 ppm).

2.3. Statistical and Genetic Analysis

All trait data were validated for normality using the Shapiro-Wilk test and checked for homogeneity of variance with Levene's test prior to analysis using SAS 9.4 [37].

2.3.1. Growth and Morphological Traits

An analysis of variance and covariance was conducted for height, diameter, and biomass traits using the following linear model:

$$Y_{ijk} = \mu + R_i + P_j + RP_{ij} + F_k(P_j) + \varepsilon_{ijk} \quad (1)$$

where

μ - grand mean
R_i - ith replication effect, i = 1-16
P_j - jth population effect, j = 1-6
RP_{ij} - effect of replication-by-population interaction
$F_k(P_j)$ - kth family effect within the jth population, k = 1, 2, …, n (n ranges from 13 to 27)
ε_{ijk} - residual error

All model effects (e.g., family, population, block) were treated as random to account for the hierarchical variance structures inherent in the experimental design. Variance and covariance components were estimated using the Type I method (sequential sum of squares) via the Varcomp procedure in SAS 9.4 [37]. This approach partitions observed variation into contributions from random effects while calculating expected mean squares (EMS) and expected mean cross products (EMCP) [38]. The resulting EMS/EMCP matrices provided a basis for deriving unbiased estimates of additive genetic variance, genotype-by-environment interactions, and residual covariance (Table 3).

Hypothesis testing for variance components relies on Satterthwaite's approximation [39], which adjusts the denominator degrees of freedom in mixed models to address unbalanced designs and unequal replication. This method is robust for nested random effects and minimizes Type I error inflation in small-sample contexts. Table 3 provides the complete structure of the analysis of variance (ANOVA) and covariance (ANCOVA), including model terms, degrees of freedom, and variance partitioning outcomes.

To validate model assumptions, residuals were inspected for normality (Shapiro-Wilk test, $W > 0.95$) and homoscedasticity (Levene's test, $p > 0.05$). All analyses were conducted at $\alpha = 0.05$, with significance thresholds adjusted for multiple comparisons using the Benjamini-Hochberg procedure in SAS [37] where applicable.

The observed inbreeding coefficients ($F = 0.15$–0.32; Table 1) indicate moderate population-level inbreeding, necessitating adjustments to variance component interpretations. Following quantitative genetic theory for partially inbred populations [40], family variance (σ^2_{fx}) was assumed to approximate one-third of the additive genetic variance due to increased homozygosity and shared maternal pedigree effects [41]. This adjustment aligns with expectations for open-pollinated progeny of tropical trees with mixed mating systems [42]. The narrow-sense heritabilities for individuals and families were computed as follows:

$$\text{Individual heritability,}\ \ h^2_i = \frac{3 \times \sigma^2_{f(p)}}{\sigma^2_e + \sigma^2_{f(p)}} \qquad (2)$$

$$\text{Family heritability,}\ \ h^2_f = \frac{\sigma^2_{f(p)}}{\sigma^2_e / k_{10} + \ \sigma^2_{f(p)}} \qquad (3)$$

Table 3 summarizes the terms mentioned above. Nyquist [43] provided the formula for estimating the standard errors of heritabilities.

Genetic correlation (r_g) between traits was calculated following Falconer [44]:

$$r_g = \frac{COV_f\,(x, y)}{\sqrt{\sigma^2_{fx}\sigma^2_{fy}}} \qquad (4)$$

Where COV_f (x,y) represents the family covariance between traits X and Y, and σ^2_{fx} and σ^2_{fy} are their corresponding family (i.e., family-within-population) variances. The standard error of genetic correlation was estimated following Robertson [45].

2.3.2. Physiological Traits

The analysis of variance and covariance for physiological traits was conducted using the following linear model:

$$Y_{ijkl} = \mu + R_i + P_j + RP_{ij} + F_k(P_j) + L_l(FP_{jk}) + \varepsilon_{ijkl} \qquad (5)$$

where

μ - grand mean

R_i - ith replication effect, i = 1-5

P_j - jth population effect, j = 1-6

RP_{ij} - effect of replication-by-population interaction

$F_k(P_j)$ - kth family effect within the jth population, k = 1, 2, …, n (n ranges from 13 to 27)

$L_l(FP_{jk})$ - lth leaf effect within the kth family within the jth population, l = 1-2

ε_{ijkl} - residual error

All effects in the model were assumed to be random. Expected mean squares (EMS) and expected mean cross products (EMCP) were estimated using SAS Varcomp procedure [37]. Table 4 presents the structure of the analysis of variance and covariance. Significance tests of the effects in the model followed Satterthwaite's approximate test procedure [39].

Similar to growth and morphological traits, the narrow-sense heritabilities for individuals and families were computed as:

$$\text{Individual heritability,}\ \ h_i^2 = \frac{3 \times \sigma^2_{f(p)}}{\sigma^2_e + \sigma^2_{l(fp)} + \sigma^2_{f(p)}} \quad (6)$$

$$\text{Family heritability,}\ \ h_f^2 = \frac{\sigma^2_{f(p)}}{\sigma^2_e / k_{14} + k_{13}\sigma^2_{l(fp)} / k_{14} + \sigma^2_{f(p)}} \quad (7)$$

Table 4 summarizes the terms mentioned above. Nyquist [43] provided the formula for estimating the standard errors of heritabilities.

The genetic correlation (r_g) between traits was calculated using Equation (4), and the standard error was determined according to Robertson [45].

3. Results

3.1. Seedling Survival

Seedling survival after the 30-week growth period remained consistently high, averaging 95% across all populations (range: 92–97%). At the family level, survival rates showed greater variability, ranging from 75% to 100% (Table 5). However, interpopulation differences in survival were not statistically significant ($p > 0.05$), indicating that environmental or management factors (e.g., uniform nursery conditions) were more influential than genetic variation in survival outcomes.

3.2. Growth and Morphological Traits

Population means, standard deviations, family mean ranges, grand means, and coefficients of variation (CV) for seedling height growth are summarized in Table 6. Height growth accelerated between weeks 6 and 18 (grand mean: 36.2 cm), followed by a gradual decline towards the end of the 30-week growth period. CV values consistently increased with seedling age, reflecting heightened phenotypic variability over time (Table 6). Population 5 exhibited the greatest final height (40.9 cm), exceeding the grand mean by 13%, while Population 1 showed the lowest mean height (29.8 cm), 17% below the grand mean. Despite both originating from mixed deciduous forests, the 27% disparity in height between Populations 1 and 5 underscores potential geographic or genetic differentiation.

Diameter growth remained relatively stable throughout the study (Table 7), with slightly higher rates observed in younger seedlings. CV values for diameter were lower than those for height, indicating less inter-seedling variability, though they increased marginally with age. Population 5 again demonstrated superior performance, boasting a final diameter of 11.04 mm (6% above the grand mean of 10.38 mm), while Population 1 lagged at 9.76 mm (6% below the grand mean). This 11% divergence between populations aligns with the trends observed in height growth (Table 7).

Biomass partitioning revealed preferential allocation to shoots over roots, with shoot biomass averaging 6.281 g (grand mean) compared to 5.094 g for roots, yielding a shoot-to-root ratio (S:R) of 1.354 (Table 8). Shoot biomass was evenly distributed between leaves (3.027 g) and stems (3.254 g), while taproots accounted for 73.6% of total root biomass (3.748 g versus 1.356 g for fibrous roots). Biomass traits exhibited CV values two to three times higher than those of height or diameter, except for specific leaf weight (SLWT), which displayed minimal variation (Tables 6–8). Population 5 produced the highest total dry weight (14.429 g; 26% above the grand mean of 11.375 g), contrasting sharply with Population 1 (8.741 g; 23% below the grand mean). Similar trends were observed across individual biomass components (Table 8).

Analysis of variance (ANOVA) for height growth (Table 10) identified highly significant population and family-within-population effects ($P < 0.01$) across all measurements. Replication effects gained significance after week 12 ($P < 0.01$), while replication-by-population interactions remained nonsignificant. Family-within-population variance dominated early growth, declining from 32% to 13% by week 30, whereas residual variance accounted for 53–69% of total variability. Similar patterns emerged for diameter growth (Table 11), with family-within-population effects explaining 16–21% of variance and residuals 72–78%. Biomass traits (Table 12) showed significant replication, population, and family effects ($P < 0.01$), except SLWT, where replication explained fivefold more variance than genetic components.

Heritability estimates for height growth ranged from 0.494 (±0.078) to 1.00 (±0.11) for individuals and from 0.744 (±0.036) to 0.895 (±0.015) for families, declining slightly with age (Table 14). Diameter heritabilities were similarly high (individual: 0.549–0.680; family: 0.768–0.812), with family estimates exceeding individual values by 32%. Biomass traits exhibited moderate heritabilities (individual: 0.386–0.564; family: 0.686–0.774), except for S:R (0.291 individual; 0.614 family) and SLWT (0.094 individual; 0.324 family), which showed reduced genetic control (Table 14).

Height growth at successive ages demonstrated strong genetic correlations (0.394–0.997), which declined over temporal intervals (Table 15). Height-diameter correlations ranged from 0.546 to 0.917 (Table 16), whereas height-biomass correlations strengthened with age, peaking at 0.892 for stem biomass. Diameter-biomass correlations reflected this trend (0.436–0.892; Table 18). Biomass traits exhibited high inter-trait correlations (0.663–0.980), except for S:R and SLWT, which correlated weakly or negatively with root components (Table 19). Shoot biomass traits (leaf, stem) demonstrated stronger integration than root traits (taproot, fibrous root).

3.3. Physiological Traits

Net photosynthesis (*A*), transpiration (*E*), and water-use efficiency (WUE) of *P. macrocarpus* seedlings averaged 8.39 μmol m^{-2} s^{-1}, 1.4 mmol m^{-2} s^{-1}, and 6.56 μmol CO_2/mmol H_2O, respectively, with significant inter-seedling variability (coefficients of variation [CV]: 18-23%; Table 9). Population 5 demonstrated the highest mean net photosynthesis (9.08 μmol m^{-2} s^{-1}; 8% above the grand mean), aligning with its superior growth and biomass performance. Although Population 1 did not exhibit the lowest *A* (7.98 μmol m^{-2} s^{-1}), it recorded the highest transpiration rate (1.49 mmol m^{-2} s^{-1}), contrasting with Population 3, which had the lowest *E* (1.31 mmol m^{-2} s^{-1}; Table 9).

Water-use efficiency varied significantly across populations. Population 5, which combined high *A* and low *E*, achieved the highest WUE (7.28 μmol CO_2/mmol H_2O; 11% above the grand mean), while Population 1, characterised by low *A* and high *E*, exhibited the lowest WUE (5.82 μmol CO_2/mmol H_2O). This 20% difference highlights the relationship between photosynthetic efficiency and water conservation strategies among geographically distinct populations.

Analysis of variance (Table 13) revealed highly significant family effects ($P < 0.01$) for all physiological traits, highlighting genetic influences on phenotypic variation. Population effects were significant only for WUE ($P < 0.01$), while replication effects were notable for *E* and WUE ($P < 0.01$). Replication-by-population interactions were significant for *A* ($P < 0.01$) and *E* ($P < 0.05$), suggesting environmental modulation of these traits. Residual variance accounted for the majority of total variability (75–83%), aligning with trends observed in growth and biomass traits (Table 13).

Heritability estimates ranged from low to moderate: individual heritabilities spanned from 0.256 (±0.079) for WUE to 0.428 (±0.092) for *E*, while family heritabilities varied between 0.473 (±0.10) and 0.615 (±0.073) (Table 14). Transpiration exhibited the highest heritability, consistent with its sensitivity to stomatal regulation mechanisms. The standard errors for these estimates (11–29%) exceeded those of growth traits, reflecting greater environmental plasticity in physiological responses (Table 14).

Net photosynthesis and transpiration exhibited a strong positive genetic correlation (0.782 ± 0.117), whereas physiological traits were only weakly linked to growth and biomass parameters (Table 20). WUE correlations with its components (A, E) were excluded due to the formulaic dependencies inherent in its calculation. WUE showed marginally stronger associations with growth traits (e.g., height, stem biomass) than A or E; however, correlations remained nonsignificant ($r < 0.3$) with large standard errors. These results indicate that physiological processes like photosynthesis and transpiration function independently of morphological growth under the studied conditions, potentially due to divergent resource allocation strategies.

4. Discussion

4.1. Nursery Performance and Early Growth Dynamics

Seedlings of *P. macrocarpus* exhibited exceptional survival rates (mean: 95%; range: 75–100%) under nursery conditions (Table 5), demonstrating their adaptability to controlled environments. The high survival rate corresponds with findings in other tropical hardwoods, where optimal resource availability in nurseries reduces abiotic stress, facilitating robust establishment and early growth [46–48]. Such conditions aid in expressing genetic variation in juvenile traits, a critical consideration for breeding programmes aimed at species with extended life cycles [46,47].

4.2. Growth and Morphological Traits

Seedlings reached a mean height of 36.2 cm, a diameter of 10.38 mm, and a total biomass of 11.375 g after 30 weeks (Tables 6–8). The significant height growth observed between weeks 6 and 18 reflects typical exponential growth phases in juvenile trees, followed by a decline post-transplantation (week 12), likely due to transplant shock and resource reallocation to root systems [49–51]. A subsequent slowdown after week 24 coincided with seasonal dormancy (January–March), during which *P. macrocarpus* sheds foliage, redirecting resources to structural and storage tissues.

In contrast, diameter growth remained stable throughout the season (Table 7), mirroring patterns in *Pinus taeda* and Douglas-fir trees where diameter increments persist even as height growth stagnates [52,53]. This indicates differential sensitivity to dormancy signals between apical and radial meristems. Late-stage reductions in diameter growth may reflect intra-seedling competition under increasing density, as spacing effects on secondary growth are well-documented [54,55].

Biomass allocation favoured shoots (S:R = 1.354), with taproots (TROOT: 3.748 g) dominating root systems over fibrous roots (FROOT: 1.356 g) (Table 8). This aligns with strategies observed in deciduous species, where taproots enhance drought resilience by accessing deeper soil moisture during dormancy [56–58]. Reduced investment in fibrous roots, which cease activity in dry seasons, may optimise survival under seasonal water stress [57,58].

Significant population-level differences emerged, with Population 5 (northern Thailand) outperforming Population 1 (western Thailand) by 27% in height, 11% in diameter, and 39% in total biomass (Tables 6–8). These disparities likely reflect local adaptation, as Population 5 originated from a site ecologically similar to the nursery, whereas Population 1 faced contrasting native conditions. Such environmental filtering parallels germination response variability in *P. macrocarpus* [59] and underscores the necessity for provenance-specific conservation strategies.

ANOVA confirmed substantial genetic variation among populations and families (Tables 10–12), consistent with studies on outcrossing tropical trees [60–64]. The predominance of within-family variance aligns with expectations for insect-pollinated species like *P. macrocarpus*, where high pollen diversity maintains heterozygosity [35]. Population differentiation exceeded that typical of conifers [65–69], likely due to fragmented habitats restricting gene flow—a phenomenon exacerbated in insect-pollinated taxa with limited pollinator mobility [70–74].

The family-within-population effect was substantial, mirroring isozyme results indicating that most genetic variation in *P. macrocarpus* resides within populations [26]. The random selection of seed trees, without bias toward superior phenotypes, may have contributed to maintaining this variation [60,75,76].

Family-level variance peaked early (13% by week 30; Table 10), reflecting genetic expression under low competition [46,62,77,78]. The decline in family effects with age mirrors competition-driven suppression of genetic signals, as observed in Eucalyptus and Pinus [77,79,80]. Maternal effects, mediated by seed weight, strongly influenced early growth (Table 21), with height correlations decreasing from 0.727 (week 3) to 0.377 (week 30), consistent with diminishing maternal provisioning impacts [81–85].

Moderate to high heritability estimates (Table 14) for height (h_i^2: 0.494–1.00; h_f^2: 0.744–0.895) and diameter (h_i^2: 0.549–0.680; h_f^2: 0.768–0.812) surpass values reported for many tropical species [86–88], likely due to controlled nursery conditions that minimize environmental noise. The decline in height heritability with age corresponds with increased competition, which masks genetic differences [32,48], while the stable heritability of diameter indicates stronger maternal or pleiotropic influences.

Biomass traits demonstrated moderate heritability (e.g., total biomass h_i^2: 0.522; h_f^2: 0.757), except for S:R (h_i^2: 0.291) and SLWT (h_i^2: 0.094), which were limited by high residual variance (Table 12). SLWT's sensitivity to microclimatic variability [94–96] highlights its restricted utility for selection, while S:R's plasticity indicates adaptive trade-offs between shoot and root investment under changing resource availability [88–92].

Strong genetic correlations among height, diameter, and biomass traits (Tables 15–19) support pleiotropic gene action, enabling concurrent selection for multiple traits. Height-diameter correlations (0.546–0.917) and biomass linkages (e.g., LEAF-STEM: 0.852) suggest integrated developmental regulation. Weak S:R and SLWT correlations with other traits highlight their environmental lability, requiring cautious interpretation [94–96].

Early-age correlations (e.g., H3-LEAF: 0.297) underscore the transient influence of maternal effects, while late-age stability (e.g., H30-TOTAL: 0.803) supports reliable selection post-dormancy [32,48,97]. The feasibility of early culling (e.g., eliminating low-performing families at week 12) aligns with cost-effective nursery management strategies [34,97,98], although field validation remains critical to account for genotype-by-environment interactions.

4.3. Physiological Traits

The net photosynthetic rate (A) in *P. macrocarpus* seedlings ranged from 7.88 to 9.08 μmol m^{-2} s^{-1} among populations, with Population 5 (northern Thailand) achieving the highest values (Table 9). Although differences between populations were non-significant (Table 13), Population 5's superior performance under nursery conditions, which closely resemble its native climate, suggests local adaptation, a phenomenon documented in *Pinus taeda* and *Eucalyptus globulus* [52,88,100,101]. Such climate-of-origin effects on photosynthesis are well established, reflecting acclimation to temperature, humidity, and light regimes [102–104].

Transpiration rate (E) exhibited similar patterns, with Population 1 (western Thailand) displaying the highest E (1.49 mmol m^{-2} s^{-1}) despite originating from a cooler native habitat (Table 9). Increased transpiration in warmer environments aligns with findings in *Eucalyptus camaldulensis*, where heat stress boosted stomatal conductance [88]. This plasticity underscores the species' capacity to adjust water-use strategies under novel conditions, although such responses may involve trade-offs in water-limited settings.

Water-use efficiency (WUE), calculated as A/E [36], was highest in Population 5 (7.28 μmol CO_2 mmol^{-1} H_2O) and lowest in Population 1 (5.82 μmol CO_2 mmol^{-1} H_2O) (Table 9). The high A and moderate E in Population 5 reflect adaptations observed in xeric *Populus trichocarpa* clones that optimize carbon gain during drought [105]. In contrast, the low WUE of Population 1, driven by high E and modest A, likely exacerbated water deficits in containerized nursery conditions, contributing to its reduced biomass accumulation (Tables 6–9). These results highlight the interplay between physiological adaptation and microenvironmental constraints in shaping seedling performance.

Significant family-within-population effects for *A*, *E*, and WUE (Table 13) highlight substantial genetic variation in gas exchange traits, consistent with patterns in growth and biomass traits. However, population-level differences were significant only for WUE, suggesting that selective pressures on water-use efficiency may operate more strongly at broader geographic scales. Comparable findings in *Picea abies* and *Alnus rubra* reveal inconsistent genetic structuring of gas exchange traits, often attributed to contrasting environmental gradients and gene flow dynamics [106–108].

The negligible population effects on *A* and *E* contrast with reports in *Larix occidentalis*, where differences in photosynthesis between provenances were absent [109]. This discrepancy may reflect the homogenizing influence of nursery conditions or the limited sampling of extreme ecotypes. Significant replication × population interactions further suggest that genetic differences in physiology are context-dependent and modulated by microenvironmental heterogeneity, such as light availability or irrigation schedules.

Narrow-sense heritability estimates for *A* (0.399 ± 0.09), *E* (0.428 ± 0.092), and WUE (0.256 ± 0.079) were moderate, with low standard errors confirming genetic control (Table 14). These values align with heritabilities reported for crops like *Zea mays* and *Triticum aestivum*, where photosynthetic traits exhibit similar environmental sensitivity [110–113]. The lower heritability of WUE compared to *E* underscores its dual dependence on stomatal regulation and carboxylation efficiency, both of which are highly responsive to diurnal and seasonal fluctuations [52,101,114].

Ontogenetic shifts further complicate the expression of physiological traits. Declining gas exchange rates with seedling age, as observed in *Prunus serotina* and *Robinia pseudoacacia* [115,116], reflect metabolic reallocations from leaf production to structural biomass. Leaf morphological plasticity, documented in *P. macrocarpus* (Liengsiri, pers. obs.), introduces additional variability, as specific leaf weight (SLWT) and longevity influence photosynthetic capacity [94,117,118]. Despite these challenges, the moderate heritabilities reported here affirm the potential for enhancing physiological traits through selective breeding, particularly in stable nursery environments.

The strong genetic correlation between *A* and *E* (0.782 ± 0.117) reflects coordinated stomatal and biochemical regulation of gas exchange, a pattern noted in *Alnus rubra* [108]. However, the weak correlations between physiological traits and growth/biomass parameters (Table 20) limit their utility as proxies for long-term productivity. For instance, while *Pseudotsuga menziesii* exhibits tight linkages between WUE and growth under drought [119], such relationships are inconsistent in *P. macrocarpus*, likely due to methodological and temporal disparities.

Gas exchange measurements capture instantaneous leaf-level processes, while growth integrates cumulative physiological performance across tissues and time [14,16,111]. Environmental heterogeneity, such as uneven light distribution or root-zone moisture, further decouples leaf-level physiology from whole-plant outcomes [120,121]. Therefore, single-point measurements of *A* or *E* provide limited predictive power for field performance, reflecting critiques in *Picea abies* and *Pinus sylvestris* [106,120].

4.4. Synthesis

This study elucidates the genetic and environmental drivers of early growth in *P. macrocarpus*, highlighting its potential for genetic improvement. However, long-term success hinges on extending trials to field conditions, quantifying G×E interactions, and integrating genomic tools to resolve adaptive loci. Prioritizing high-WUE genotypes, as suggested by physiological data, could enhance drought resilience in reforestation programs, a pressing need given Thailand’s escalating deforestation and land degradation. Collaborative efforts between nurseries and conservation agencies will be essential to translate these insights into scalable, ecologically informed strategies for sustainable forest management.

While this study confirms the genetic basis of physiological variation in *P. macrocarpus*, several constraints warrant attention. First, containerized nursery conditions may exaggerate water stress, skewing WUE estimates compared to field settings. Second, focusing on juvenile seedlings overlooks ontogenetic shifts in gas exchange, which may alter trait rankings at maturity [115,116]. Finally, lacking genotype-by-environment (G×E) interaction data limits extrapolation to diverse planting sites.

Future research should prioritise multi-environment trials to quantify G×E effects and identify stable, high-performing families. Integrating genomic tools (e.g., QTL mapping, transcriptomics) could clarify the genetic architecture of WUE and its component traits, thus accelerating marker-assisted selection. Additionally, longitudinal studies tracking physiological and growth trajectories from nursery to field will enhance the predictive value of early-stage measurements.

5. Conclusion

This study highlights the substantial genetic variation and heritable control underlying growth, biomass accumulation, and physiological traits in *P. macrocarpus* seedlings, reinforcing the species' potential for targeted genetic improvement and early selection in breeding programs. The heritability of these traits, evaluated over one growing season (6.5 months), offers critical foundational insights into the genetic architecture of seedling performance. However, the temporal and environmental limitations of this investigation emphasize the need to extend evaluations to mature developmental stages and field conditions. Such longitudinal studies are vital to clarify age-dependent genetic dynamics, changes in trait heritability throughout ontogeny, and the modulating effects of environmental stressors on phenotypic expression.

Given *P. macrocarpus*'s broad natural distribution and documented population differentiation across heterogeneous landscapes [26], future trials must rigorously incorporate multi-site experimental designs to quantify genotype-by-environment (G×E) interactions. These data are essential for delineating breeding zones, defining conservation units, and optimizing deployment strategies to align genetic resources with site-specific ecological conditions. Furthermore, the species' deciduous phenology and interannual leaf morphological plasticity (Liengsiri, personal observation) necessitate repeated, multi-season assessments under various environmental regimes to yield robust estimates of physiological trait stability. Among these traits, water-use efficiency (WUE) emerges as a particularly promising candidate for selective breeding. Despite the labour-intensive nature of physiological measurements, integrating WUE into selection criteria could enhance seedling resilience in drought-prone or degraded habitats, offering practical benefits for reforestation and ex-situ conservation initiatives.

In Thailand, where extensive deforestation has led to land degradation characterized by decreased soil fertility, reduced hydrological retention, and significant microclimatic fluctuations, establishing seedlings remains a critical challenge. While silvicultural interventions, such as site preparation and post-planting irrigation, can enhance early survival rates, their economic costs often hinder large-scale implementation. Prioritizing genotypes with elevated WUE presents a scalable, cost-effective strategy for boosting seedling productivity under water-limited conditions. This approach aligns with established evidence that WUE is a key determinant of plant performance in arid environments [122,123], underscoring its utility in breeding programmes aimed at ecological restoration.

Notably, this study also reveals potential inbreeding effects on growth performance. Families exhibiting reduced outcrossing rates displayed significantly diminished growth trajectories (Table 1), consistent with prior evidence of inbreeding depression in *P. macrocarpus* populations. Such depression, widely documented in forest trees [124], risks introducing bias into estimates of additive genetic variance and heritability [41,42]. To mitigate these effects, breeding programs must adopt strategies that explicitly minimize inbreeding, such as controlled crossing designs or the maintenance of diverse founder populations, thereby ensuring the accuracy of selection and the long-term viability of genetic gains.

In conclusion, while these findings confirm the genetic potential of *P. macrocarpus* for trait improvement, the success of long-term breeding and conservation efforts depends on ongoing research into three interconnected pillars: (1) spatiotemporal patterns of genetic diversity throughout the species' range, (2) the scale and drivers of G×E interactions within diverse landscapes, and (3) the physiological mechanisms underlying drought adaptation and resilience. Addressing these priorities will enable evidence-based strategies for germplasm conservation, precision breeding, and adaptive silviculture. By integrating genetic, environmental, and physiological insights, stakeholders can optimize the ecological and economic value of *P. macrocarpus* in an era of increasing climatic uncertainty, ensuring its continued role in restoring degraded ecosystems and supporting sustainable forest management.

6. Recommendations for Further Study

I. Pan-Regional Genetic Diversity Assessment

To enhance the applicability of findings to regional conservation and breeding programs, future studies should broaden the genetic variation analyses of *P. macrocarpus* to include its entire natural distribution, incorporating populations beyond Thailand. A comprehensive understanding of rangewide genetic structure is crucial to developing effective strategies for conserving genetic resources and breeding. Furthermore, parallel investigations into sympatric taxa of ecological and economic significance could clarify shared evolutionary pressures and inform holistic management strategies for co-occurring species complexes.

II. Fine-Scale Spatial Genetic Structuring

Spatially explicit genetic analyses within populations are encouraged to quantify fine-scale genetic structure. This research will clarify the spatial clustering of related individuals, facilitating the development of optimized sampling protocols that reduce kinship bias during germplasm collection. This approach will ensure that conservation and breeding programs maximize genetic diversity while preserving population integrity.

III. Temporal Dynamics of Mating Systems

Longitudinal studies that evaluate interannual variation in mating systems—ideally within the same populations and parent trees—are essential for refining seed harvesting guidelines. Determining whether outcrossing rates and pollen dispersal patterns fluctuate with flowering intensity could help inform protocols for the timing of seed collection to optimise the capture of genetic diversity. For instance, prioritising mast years or high-flowering cohorts may reduce losses in genetic variation when sampling limited maternal trees.

IV. Genotype-by-Environment Interaction Analysis

Expanding quantitative genetic studies through multi-location trials conducted over multiple years is essential for quantifying genotype-by-environment (G×E) interactions in *P. macrocarpus*. Such data are indispensable for delineating breeding and deployment zones, ensuring that genotypes align with optimal ecological conditions. This research would also clarify adaptive potential under climatic variability, directly supporting the sustainable management of genetic resources.

V. Drought Adaptation Mechanisms in Seedlings

Investigations into morphological and physiological responses to water stress, particularly the plasticity of dry matter allocation between root and shoot systems, are crucial for enhancing silvicultural practices. While foliar acclimation may provide short-term drought tolerance, root architectural adaptations can deliver lasting resilience, vital for seedling survival in water-limited environments. Clarifying these trade-offs will improve nursery protocols and reforestation strategies for arid or seasonally dry habitats.

These priorities collectively address knowledge gaps in genetic resource management, adaptive capacity, and ecological resilience, offering actionable pathways to protect *P. macrocarpus* and its associated ecosystems amidst shifting environmental pressures.

Author Contributions: Conceptualization: LC and FCY; Methodology: LC and FCY; Data Collection: LC; Data Curation: LC and FCY; Validation: LC and FCY; Formal Analysis: LC; Writing—Original Draft Preparation: LC;

Writing—Review and Editing: LC and FCY; Funding Acquisition: FCY; LC and FCY have read and agreed to the published version of the manuscript.

Funding: This research was partially funded by grants from the Natural Sciences and Engineering Research Council of Canada, STR0118500 and A2282 to FCY.

Data Availability Statement: The original contributions presented in this study can be directed to FCY.

Acknowledgments: LC and FCY thank the editor and reviewers for their helpful feedback in improving this paper. LC expresses gratitude to the Royal Forest Department, Thailand, and colleagues at the ASEAN Forest Tree Seed Centre for their support; FCY acknowledges the resources provided by the Department of Renewable Resources, University of Alberta.

Conflicts of Interest: LC and FCY declare that they have no conflicts of interest.

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