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Brief Report

Assessing 62 Chinese Fir (*Cunninghamia lanceolata*) Breeding Parents in a 12-Year Grafted Clone Test

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Abstract: Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) is one of the major commercial conifer species in China. The present study concentrated on the assessment of growth, wood property traits, and strobili number in a 12-year grafted clone test of 62 Chinese fir breeding parents, aiming to describe the variation and correlations between these traits and to identify parent clones with the highest potential for future breeding. The results indicate that all of the growth (height, diameter at breast height, stem volume, crown-width) and wood property (wood basic density and hygroscopicity) traits varied significantly ($p < 0.01$) among clones, with coefficients of variation ranging from 7.6% to 30.6%. Furthermore, these traits consistently had a moderate to high (0.39–0.87) repeatability estimate (broad-sense heritability). Remarkable clonal differences were also observed for the production of male and female strobili. Phenotypic correlations among growth traits were strong ($p < 0.01$) and positive. Significantly negative correlations ($p < 0.01$ or 0.05) were found between wood basic density and growth (except for height) and hygroscopicity. The production of male and female strobili appeared to be significantly ($p < 0.01$) positively correlated with each other. A notable number of faster-growing parent clones were identified ($n = 30$); 11 of these had higher density wood with an average realized gain of 10.5% in diameter, and a 5.4% gain in wood basic density. When selection was made for growth and strobili, 10 faster-growing parent clones with medium to high production of female strobili were identified.

Keywords: *Cunninghamia lanceolata*; clone test; repeatability; correlation; realized gain

1. Introduction

Conifers dominate approximately 39% of world forests and play an important role in forestry-dependent economics and global ecosystems (e.g., carbon capture, the diversity of native habitat, and erosion control/water quality) [1,2]. They comprise 69 genera, representing approximately 614 known species [3–5]. A subset of conifers has been used as breeding subjects with a goal to enhance useful traits, such as rapid growth, high wood quality, and biotic and/or abiotic resistance. Representatives of these breeding subjects include loblolly pine (*Pinus taeda* L.) [6], radiata pine (*Pinus radiata* D. Don) [7], Norway spruce (*Picea abies* (L.) Karst.) [8], and Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) [9], *etc.* Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook), an evergreen coniferous tree that occupies approximately 25% of plantations in southern China, has received a considerable breeding effort over the past 50 years, largely due to its great afforestation values and ecological contributions [10,11].

Similar to other conifer breeding programs around the world, the improvement of Chinese fir mainly emphasizes the enhancement of growth and adaptability with the following activities: provenance testing, plus-tree selection, establishment of breeding garden/gene bank, family/progeny (half-sib and full-sib) and clonal testing, parent tree (plus-tree clone) reselection, clonal seed orchard establishment, and so forth [12]. Wood quality traits were recently targeted as a breeding objective due to increasing demands for high-quality timber in the last 10 years. To advance the multi-trait breeding process, it is essential to profile the breeding parents/materials using either clonal testing or progeny trials, of which, clonal testing has proven to be a straightforward and effective strategy [13–16]. In the present study, to identify the variation and correlation in growth, wood property traits, and strobili number, 62 Chinese fir breeding parents were subjected to a 12-year grafted clone test with an eventual goal to select the best potential parent clones for future breeding programs.

2. Experimental Section

This work was carried out in the breeding garden located at Longshan State Forest Farm, Guangdong, China (25°11′ N, 113°28′ E, 285–296 m above sea level). Sixty-two Chinese fir plus-trees (breeding parents) selected for second/third generation breeding programs of different provinces in China (Guangdong (n = 15; p1, p4, p5, p6, p7, p136, p156, p169, p173, p207, p210, p214, p234, p256, and p712), Fujian (n = 14; P94, P95, p96, p97, P98, P99, P100, p101, p102, p103, p104, p271, p399, and p710), Guangxi (n = 13; p18, p20, p21, p22, p148, p241, p254, p286, p294, p298, p302, p305, and p346), Guizhou (n = 11; p237, p238, p243, p247, p252, p258, p265, p272, p285, p711, and p713), Hunan (n = 8; p8, p9, p15, p16, p17, p25, p26, and p226), and Anhui (n = 1; p105)) were tested herein. The plus-tree scions were synchronously grafted onto 2-year-old rootstocks with a 1-tree plot randomized distribution design (6–12 ramets per clone) and a 5 × 5-m spacing since 2002. Trees were maintained through standard commercial practices.

All trees were measured for traits of height (H), diameter (diameter at breast height (1.3 m), DBH), and crown-width (CW) in 2007 (6 years old), and for DBH in 2013 (12 years old). The tree stem volume (V) was calculated according to the formula $V = 5.8777042 \times 10^{-5} \times DBH^{1.9699831} \times H^{0.89646157}$ [15]. The abundance of strobili (male or female) was evaluated using a grading method during 2007–2013, with a record of high (coded as 5: many strobili on over 70% of branches), medium (coded as 3: a considerable number of strobili on over 50% of branches), or low (coded as 1: sparse strobili on fewer than 50% of branches) for each tree.

Each parent clone had at least four ramets of similar size and vigor at age 12. Three randomly selected ramets were measured for wood basic density (WBD) and hygroscopicity (Hy) with an increment core method described by Zheng *et al.* [11]. In brief, a 5.02-mm increment core was taken at breast height (1.3 m) from each tree using a tree growth cone. WBD (g/cm^3) was estimated using the formula $WBD = W_2/(W_1 - W_2 + W_2/\rho_{cw})$, where W_1, W_2 and ρ_{cw} represent water-saturated weight, oven dry weight and wood cell wall component density, respectively; we used the constant 1.53 g/cm^3 for ρ_{cw}, and the present formula can be simplified as $WBD = 1/((W_1/W_2) - 0.346)$ [17]. Hygroscopicity (Hy) was evaluated using the formula $Hy = (W_1 - W_2)/W_2$, where W_1 and W_2 are the same as mentioned above [18].

Data were collated in Microsoft Excel 2010, and subjected to one-way analysis of variance (ANOVA) in SAS V 8.1 (SAS Institute, Cary, NC, USA) for the detection of trait differences among clones (F-value and coefficient of variation (CV)). Clonal Repeatability (R) for each trait was calculated with the formula: $R = 1 - 1/F$. The phenotypic correlation coefficient for each pair of traits was assessed with SAS PROC CORR. Of these assessments, correlations among growth and wood property traits were based on individual data (df = 184); the relationships of strobili number with other traits were based on the clonal means (df = 60). Realized gain (G) was estimated by the formula: $G = (\bar{X}_i - \bar{X})/\bar{X} \times 100\%$, where $\bar{X}_i$ and $\bar{X}$ represent the i parent clone and overall trait mean, respectively.

3. Results

3.1. Variation among Parent Clones

Differences in growth and wood property traits ($p < 0.01$) were observed for the Chinese fir parent clones ($n = 62$) at age 6 and/or 12 (Table 1). Six-year-old clones displayed a substantial variation in stem volume (V) with more than 2 times the coefficient of variation (CV) of the other growth traits (height, diameter at breast height (DBH) and crown-width (CW)); while the estimated CVs for wood properties (wood basic density (WBD) and hygroscopicity (Hy)) appeared to be higher than that of DBH at age 12. Further estimation revealed that all of the examined traits had moderate to high (0.39–0.87) repeatability (broad-sense heritability), indicating the possibility of selection of the varieties in clones.

Table 1. Mean, range, variance and repeatability of the morphological traits of Chinese fir parent clones. H, height; DBH, diameter at breast height; V, stem volume; CW, crown-width; WBD, wood basic density; Hy, wood hygroscopicity; *F*-value, ANOVA *F* (** $p < 0.01$); *CV*, coefficient of variation; *R*, repeatability.

Age	Trait	Mean ± SD	Range	*F*-value	*CV* (%)	*R*
6-year-old	H (m)	6.4 ± 0.8	5.0–8.2	2.41 **	13.3	0.59
	DBH (cm)	13.1 ± 1.7	8.1–17.4	3.38 **	11.9	0.70
	V (m^3)	0.0520 ± 0.0158	0.0185–0.0956	2.87 **	30.6	0.65
	CW (m)	3.5 ± 0.4	2.7–4.3	2.13 **	13.3	0.53
12-year-old	DBH (cm)	21.0 ± 2.6	15.4–25.7	7.68 **	7.6	0.87
	WBD (g/cm^3)	0.2752 ± 0.0210	0.2332–0.3262	1.65 **	10.2	0.39
	Hy (%)	302.3 ± 27.5	241.4–364.7	1.76 **	11.8	0.43

During 2007–2013, the growth of male and female strobili differed considerably among the 62 clones (high, medium, and low; Table 2). Most clones produced a limited number (low) of male and/or female strobili. Of the 62 clones, only 14 exhibited medium to high numbers of female strobili, and only 5 clones had fertile male strobili. Strikingly, only 1 clone (p7) had abundant numbers of male and female strobili.

Table 2. Frequencies of Chinese fir parent clones with different strobili numbers.

Level	Male Strobili	Female Strobili	(Male + Female) Strobili
High	5	1	1
Medium	13	13	7
Low	44	48	41
Total	62	62	49

3.2. Phenotypic Correlations among Traits

Positive or negative correlations were found between growth traits (H, DBH, V and CW) and wood properties (WBD and Hy) across ages 6 and 12 (Table 3). Strong positive correlations ($p < 0.01$, df = 184) were observed for the growth traits over ages 6 and 12, and significant ($p < 0.01$ or 0.05, df = 184) positive correlations were observed between Hy and growth (except for H). Notably, the WBD had significant negative correlations ($p < 0.01$ or 0.05, df = 184) with growth (except for H) and Hy at age 6 and/or 12. Male strobili production seemed to be unrelated to the wood property traits, but had positive (nonsignificant) correlations with growth. Female strobili production was also positively correlated to the growth traits, particularly for 6-year DBH ($r = 0.2566$, $p < 0.05$, df = 60). Interestingly, the production of male and female strobili was significantly positively correlated ($r = 0.6322$, $p < 0.01$, df = 60).

Table 3. Phenotypic correlations among morphological traits in Chinese fir. H-6, tree height at age 6; DBH-6, diameter at breast height at age 6; V-6, stem volume at age 6; CW-6, crown-width at age 6; DBH-12, diameter at breast height at age 12; WBD-12, wood basic density at age 12; Hy-12, wood hygroscopicity at age 12. MS, the number of male strobili; FS, the number of female strobili. * $p < 0.05$, ** $p < 0.01$. The degrees of freedom (df) for the correlations between growth and wood property traits were 184, compared with 60 when calculating the correlation coefficients among strobili number and other traits.

	H-6	DBH-6	V-6	CW-6	DBH-12	WBD-12	Hy-12	MS	FS
H-6	1.0000								
DBH-6	0.6191 **	1.0000							
V-6	0.8055 **	0.9383 **	1.0000						
CW-6	0.3432 **	0.5043 **	0.4736 **	1.0000					
DBH-12	0.3931 **	0.6826 **	0.6448 **	0.4003 **	1.0000				
WBD-12	−0.0688	−0.1770 *	−0.1582 *	−0.1466 *	−0.2683 **	1.0000			
Hy-12	0.0782	0.1862 *	0.1706 *	0.1444 *	0.2798 **	−0.9814 **	1.0000		
MS	0.1320	0.1866	0.1649	0.1340	0.1508	−0.0002	−0.0017	1.0000	
FS	0.0091	0.2566 *	0.1846	0.2367	0.2072	−0.1172	0.1390	0.6322 **	1.0000

3.3. Parent Clone Reselection

The described 62 Chinese fir clones expressed substantial variation and repeatability in both growth and wood property traits, which provides an opportunity to select faster-growing parent clones with higher wood density. Selection for growth and WBD was conducted using a two-dimensional cluster method with 12-year overall means (DBH and WBD) as thresholds (Figure 1). A total of 30 parent clones had a higher DBH growth rate than the overall mean (21.0 cm); 11 of these (p6, p8, p9, p16, p17, p95, p148, p214, p238, p247, and p271) showed higher WBD than the overall mean (0.2752 g/cm^3). The 30 faster-growing selections provided an average realized gain (G) of 10.5% in DBH and moderate increases in Hy (G = 3.1%), but tended to have lower WBD values (G = −2.5%) (Table 4). Constraining selection for WBD (>0.2752 g/cm^3) resulted in a gain of 10.5% for DBH and considerably increased the WBD, with a gain of 5.4%, although a reduction in Hy (G = −6.4%) was observed.

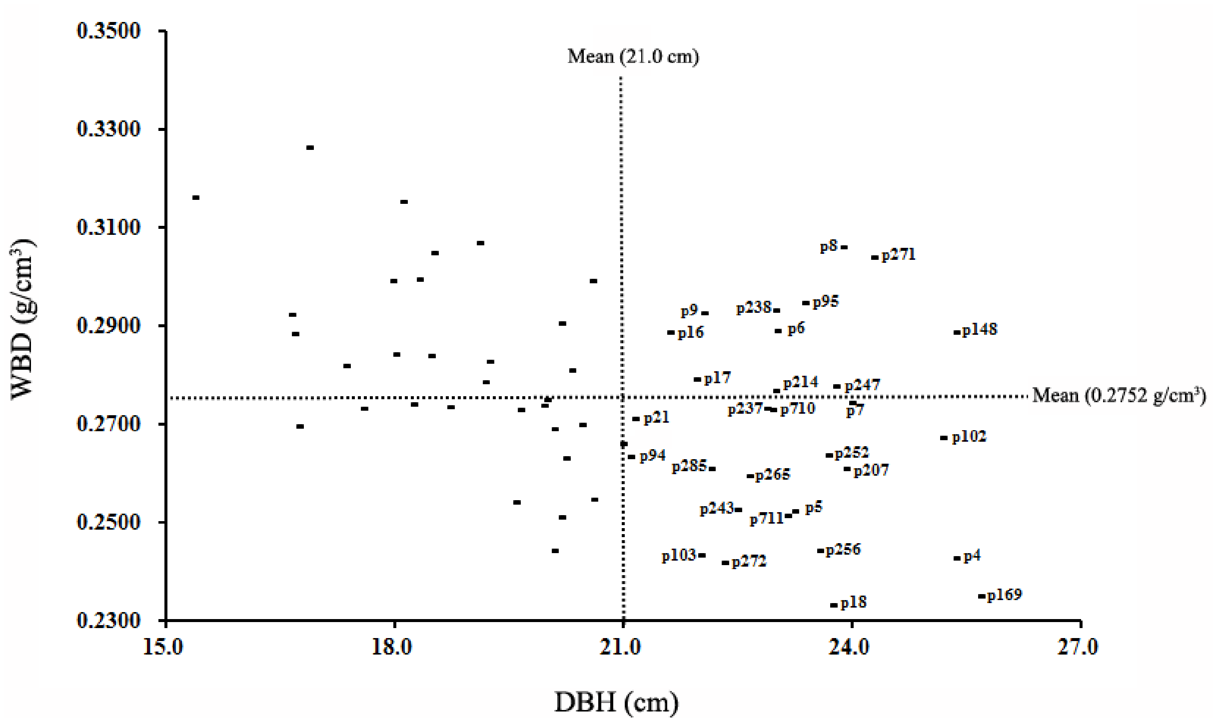

Figure 1. Two-dimensional clustering of the Chinese fir parent clones. DBH, diameter at breast height; WBD, wood basic density. The 12-year overall means were employed as a threshold (DBH and WBD); only the faster-growing clones (DBH > 21.0 cm) are marked.

Conifer breeding commonly concentrates on the selection of trees for growth and flowering. When selection was placed on DBH and the production of female strobili, 10 faster-growing parent clones (p4, p5, p6, p7, p18, p237, p238, p243, p272, and p285) were identified; this selection demonstrated a gain of 10.5% for DBH, but resulted in a loss of WBD (G = −5.1%).

Table 4. Responses in DBH, WBD and Hy when different selection criteria were used for the 62 Chinese fir breeding parents at age 12. DBH, diameter at breast height; WBD, wood basic density; Hy, wood hygroscopicity; *G*, realized gain. * Selection was carried out in the 62 Chinese fir breeding parents using the overall mean of DBH and/or WBD as a threshold; production of female strobili was at medium to high levels.

Trait	* Selection for DBH (*n* = 30)		* Selection for Both DBH and WBD (*n* = 11)		* Selection for Both DBH and Production of Female Strobili (*n* = 10)	
	Mean ± SD	*G* (%)	Mean ± SD	*G* (%)	Mean ± SD	*G* (%)
DBH (cm)	23.2 ± 1.2	10.5	23.2 ± 1.1	10.5	23.2 ± 0.9	10.5
WBD (g/cm^3)	0.2684 ± 0.0201	−2.5	0.2899 ± 0.0097	5.4	0.2613 ± 0.0205	−5.1
Hy (%)	311.6 ± 27.9	3.1	282.9 ± 12.4	−6.4	322.1 ± 29.1	6.6

4. Discussion

Regardless of the stringency of selection of plus-trees (parent tree) for advanced breeding programs, it is still necessary to employ field trials to evaluate performance superiority. Based on a 12-year grafted clone trial, variations in growth, wood properties, and flowering traits were characterized in 62 Chinese fir parent clones, and significant correlations were identified between specific traits (Table 3). Of note, this study led to the identification of a set of faster-growing parent clones, some with high density wood, and some with medium to high production of female strobili.

Grafting is the most practical way to produce elite tree clones for conservation and breeding. It is also employed as a straightforward method to obtain a recurring tree phenotype, thereby providing evidence for a genetic effect in traits [19]. Unfortunately, rootstock and graft compatibility effects on growth can be detrimental. It is worth mentioning that the grafting success in this experiment was high (>90%), and graft incompatibility was not observed in Chinese fir. Furthermore, most of the ramets remained vigorous throughout the entire study.

Genetic parameters vary with different populations, environments, and ages [20]. Bian *et al.* [12] reported that the Chinese fir repeatability of H, DBH and WBD traits in clonal tests ranged from 0.63–0.79, 0.49–0.78, and 0.51–0.75, respectively. Our report showed a high estimated repeatability for these traits (Table 1). Moderate to high repeatability indicates that selection was effective and influenced by the environment to a lesser extent [21]. Compared with the repeatability of the growth traits at age 6 (0.53–0.70), the repeatability estimate for DBH (0.87) was higher at age 12. Additionally, 12-year DBH appeared to be significantly ($p < 0.01$, df = 184) correlated with growth traits at the 6-year stage. These findings suggest that 12-year DBH could be used as a reliable criterion for the selection of faster-growing clones.

Basic density (WBD) is the most important wood property that affects nearly all wood end-product quality [22,23]. Rapid growth with higher WBD seems to be a more attractive feature for both breeders and stakeholders, as it increases the wood yield and the value for all applications [24]. Unfortunately, in most cases, correlations between WBD and growth traits appear to be negative [12], implying that increasing the WBD may, to a certain extent, result in losses in growth traits. In this study, we also found negative correlations between WBD and growth traits, and in addition, strong negative relationships between WBD, DBH and V were in agreement with the investigations reported by Li *et al.* [25] and Hu *et al.* [26,27]. Thus, care should be taken regarding selection for growth and WBD among the present parent clones. Using the overall means of 12-year DBH and WBD as a threshold, 11 faster-growing parent clones with a higher density wood were identified. They gained 10.5% in DBH and 5.4% in WBD, suggesting that these clones could be prioritized for further breeding projects.

Hygroscopicity (Hy) was another wood trait tested in this experiment because it affected multiple agent treatments on end-products [28]. Although this trait was not included in the selection criteria, it was measured throughout the selection process. When DBH was selected as a single trait or combined with female strobili, 3.1% and 6.6% gains, respectively, were observed in Hy, but reductions (−6.4%) were seen when DBH and WBD were selected together. These responses may be due to the positive correlation between Hy and growth, and the negative relationship between Hy and WBD (Table 3).

In this report, most of the studied parent clones produced a limited number of male and female strobili. One possible reason is that these parent clones originated from plus-trees selected for phenotypic vigor and adaptability, not for flower abundance. Interestingly, Nikkanen *et al.* [29] showed a significant positive relationship between strobili number and growth traits among 67 Norway spruce (*P. abies*) parent clones. Similarly, Sivacioglu *et al.* [30] reported that strobili number had a significant positive association with growth traits among 30 Scots pine (*P. sylvestris*) clones. In this study, a positive correlation between strobili number and growth traits in 62 Chinese fir parent clones was also observed, but at a nonsignificant level (except for female strobili number with 6-year DBH). However, larger testing populations are required to address the relationship between flowering and other traits. Since one of the main objectives of selection in trees is to establish seed orchards for the production of genetically improved seeds, it is of interest to select for faster-growing parent clones together with flowering. It is promising that a set of parent clones yielded medium to high numbers of female strobili, 10 of which (p4, p5, p6, p7, p18, p237, p238, p243, p272, and p285) were part of the faster-growing set. These parent clones in particular provide a significant advancement in breeding of Chinese fir, and should be prioritized for use in the production of a seed orchard.

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Author Contributions

Huiquan Zheng is the lead author. He conducted most of the field trial work and statistical analyses and wrote the manuscript. Dehuo Hu designed the experiments, and directed the estimation of the breeding garden. Runhui Wang, Ruping Wei and Shu Yan participated in the investigation of the trees.

Conflicts of Interest

The authors declare no conflict of interest.

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