

Article

# Inheritance and Correlation Analysis of Pulpwood Properties, Wood Density, and Growth Traits of Slash Pine

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**Abstract:** Slash pine (*Pinus elliottii*) is the most important and a fast-growing material that is used for industrial timber and pulp production. A breeding program of slash pine that aims to improve wood properties has been employed for the past decade. This study analysed the genetics and correlation of growth traits and wood properties of a total of 1059 individual plants from 49 families of *P. elliottii*. Heritability, family ranking, genetic gain, and the relationship between these traits were estimated. The results showed that there was a significant negative genetic correlation between the holocellulose and lignin content. The heritabilities of these four traits were ranked from 0.18 to 0.32. The chemical wood traits did not show a strong correlation with diameter at breast height (DBH) and wood density. However, it is still possible to combine wood traits for selection. It was suggested that the genetic breeding selection could improve the growth and quality of *P. elliottii*.

**Keywords:** *P. elliottii*; heritability; family ranking; pulpwood properties; wood density; DBH

## 1. Introduction

Wood is a renewable and eco-friendly material that has widely been used in construction and pulp production [1]. Wood density (WD), lignin, and holocellulose content (HC) are dominant properties for such uses. For example, the content of holocellulose plays an important role in the evaluation of pulp yield [2,3]. Lignin is a key factor that influences the pulp and paper industry. The greater the lignin content, the greater the energy consumption and pollution during the pulp production process [4,5]. However, lignin in plant cell walls could highly increase timber stiffness, water resistance, and chemical resistance. In addition, lignin in wood is also highly associated with natural durability [6,7]. WD is a critical physical property of wood quality and is strongly correlated with other wood properties, such as flexural stiffness, strength, and fiber quality [8,9]. Therefore, wood density is usually considered a vital feature in many industrial applications [10]. For instance, wood density is the main contributing factor to reduce mortality caused by broken stems and uprooted trees in bad weather [11] and it plays a very important role in pulp yield, transportation cost, and paper quality [12]. In the early stage, wood density is low in order to achieve fast height growth, and in the later stage, wood density increases in order to maintain structural stability [13]. Certainly, in the process, significant differences in the ability to resist environmental stress could be present in different genetic backgrounds.

The consistence of wood properties is needed for many industrial applications. It could vary within tree species. However, genetic selection could be an efficient way to reduce the variation. To get a better output of the genetic gain, the size of the population in a breeding program should be carefully considered [14]. A larger population size could yield greater genetic diversity and more genetic gain in a long-term experiment. However, it could also be time and cost consuming to manage a large

population. On the other hand, the selection effects strongly rely on the relationship of different wood properties and growth traits when combining several traits for selection. It would be possible to combine two or more traits only based on a large breeding population.

Slash pine (*P. elliotii*) trees, originally from the United States and widely planted in China, are the most important and a fast-growing material used for industrial timber and pulp production [15,16]. The growth and form characters have been studied, but most of the pine tree breeding studies in China have access to a small population [17] or a single wood property [18], which limits genetic improvement. In particular, few studies were done to address the pulpwood qualities of slash pine in China. To get a better understanding of the genetic basis of wood properties and to find an appropriate breeding strategy for *P. elliotii* improvement, it is critical to include a large population of genotypes and multiple wood properties.

In this study, the influence of quantitative genetics on the variability of wood lignin, HC, and density that are associated with the industry and pulp yield was examined. A total number of 1059 trees from 49 half-sibling families, which were planted in the Zhejiang province of Southern China, were conducted with the aims (1) to understand the quantitative genetics of wood properties, (2) to evaluate the genetic parameters on growth and wood properties, and (3) to examine the genetic relationship between growth traits and wood traits, especially pulpwood quality traits.

## 2. Materials and Methods

### 2.1. Materials

All of 49 half-sib families with 6 replications and 6 individuals trees of *Pinus elliotii* were planted in a randomised complete block of a 2 m × 3 m spacing at the Changle tree farm in the Yuhang district of Zhejiang, China (30°27' N, 119°48' E) as progeny trials in 1994. Trials were thinned and pruned at age 15.

### 2.2. Sample Collection

Samples were collected in November 2018, coring all living trees from the half-sibling families with a battery-powered 14-mm inner-diameter increment corer. Bark-to-bark stem cores through the pith were taken about 1 m above the ground. Stem diameter at breast height (DBH) was measured in the same time. In total, 1059 trees from 49 families were cored at the tree farm.

### 2.3. Chemical Analysis

#### 2.3.1. Lignin Content

Lignin is an aromatic macromolecular compound composed of basic structural units of phenylpropane, which has a strong UV characteristic absorption peak between 200 and 300 nm. Therefore, the lignin content was detected using UV spectrophotometry [19]. After being fully dried, the slash pine core was ground into powder, dissolved in 25% bromoacetyl-acetic acid solution, and sealed in a water bath at 70 °C for 0.5 h. Then 0.9 mL 2 mol/L NaOH was added to stop the reaction. A total of 3 mL acetic acid and 0.1 mL 7.5 mol/L hydroxylamine hydrochloric acid were further added, and the mixture was thoroughly shaken. After centrifugation for 15 min, the supernatant was placed under UV spectrophotometry at 280 nm to measure the absorption value. The optical density value was used to represent the lignin content. The experiment was repeated 3 times. The determination of lignin in plants using UV spectrophotometry is characterised by good repeatability, less sample consumption, simplicity, and rapidity.

#### 2.3.2. Holocellulose Content (HC)

The HC was measured by a modified nitric acid-ethanol method [20]. Firstly, the moisture content (W %) of the sample was measured. The nitric acid and ethanol were mixed together in a ratio of 4:1

and were kept in brown bottle for later use. Then, ~1 g of sample powder was placed in a conical bottle, 25 mL of mixed nitric acid and ethanol solution was added into the conical bottle, and stirred well, then the conical bottle was installed in a condenser and heated up to 100 °C for 70 min. The solvent was removed with a glass sand core funnel. This process was repeated 3–5 times. Then the glass sand core funnel with residue was transferred to an oven at 105 °C to get a consistent residue mass ( $M_1$  g), and the residue was placed in a crucible at 500 °C until the mass was stable and measured again ( $M_2$  g). Then the HC was calculated as:

$$\text{HC} = \frac{M_1 - M_2}{M(1 - W)} \times 100\% \quad (1)$$

where HC indicates holocellulose content (%),  $M_1$  is the total mass of holocellulose and the glass filter (g),  $M_2$  is the mass of the glass filter (g), the mass of slash pine sample is expressed in  $M$  (g), and the moisture content in  $W$  (%).

### 2.3.3. Wood Density

Considering that there are many pores on the surface of the slash pine core, an improved method based on Archimedes principle was used, which combined the hydrostatic weighing method and the sedimentation method [21]:

$$\rho = m_1 / (m_1 - m_2) \quad (2)$$

where  $\rho$  is the wood density of slash pine,  $m_1$  is the mass of the sample in the air, and the initial mass of the sample immersed in water is expressed by  $m_2$ .

### 2.4. Statistical Analysis

The details for the estimation of genetic parameters have been previously described by [22]. The restricted maximum likelihood (REML) analysis linear mixed models were used for the estimation of genetic parameters. The model in Equation (3) for a single-trait observation  $y_i$  for a tree was shown as Equation (3):

$$y_i = x_i m + b_i + f_i + e_i \quad (3)$$

where  $x_i$  is a vector linking the fixed effects  $m$  to the observation and  $b_i$ ,  $f_i$ , and  $e_i$  are the random block, family, and residual effects. Stacking those vectors for all trees produces model Equation (4):

$$y = X m + Z_1 b + Z_2 f + e \quad (4)$$

where  $y$  is a vector of phenotypic observations,  $m$  is the vector of fixed effects (overall mean), and  $b$ ,  $f$ , and  $e$  are vectors of bivariate random effects for block, family, and residual effects.  $X$ ,  $Z_1$ , and  $Z_2$  are the incidence matrices linking observations to the appropriate effects. We defined the vector of expected values ( $E$ ) and dispersion matrices ( $\text{Var}$ ) as:

$$E[y] = X m \quad (5)$$

$$\text{Var}[b] = Z_2 \otimes B_0 \quad (6)$$

$$\text{Var}[f] = Z_2 \otimes F_0 \quad (7)$$

$$\text{Var}[e] = Z \oplus R_0 \quad (8)$$

and

$$B_0 = \begin{bmatrix} \sigma_{b1}^2 & \sigma_{b1b2} \\ \sigma_{b1b2} & \sigma_{b2}^2 \end{bmatrix} \quad (9)$$

$$F_0 = \begin{bmatrix} \sigma_{f1}^2 & \sigma_{f1f2} \\ \sigma_{f1f2} & \sigma_{f2}^2 \end{bmatrix} \quad (10)$$

$$R_0 = \begin{bmatrix} \sigma_{e1}^2 & \sigma_{e1e2} \\ \sigma_{e1e2} & \sigma_{e2}^2 \end{bmatrix} \quad (11)$$

where  $\otimes$  and  $\oplus$  are the direct product and direct sum operations respectively,  $\sigma_{b_i}^2$ ,  $\sigma_{f_i}^2$ , and  $\sigma_{e_i}^2$  represent the block, family, and residual variances for trait  $i$ , and  $\sigma_{b_i b_j}^2$ ,  $\sigma_{f_i f_j}^2$ , and  $\sigma_{e_i e_j}^2$  are the covariances of block, family, and residual between traits  $i$  and trait  $j$ .

The variance components from the model were used to calculate the narrow sense heritability ( $h^2$ ):

$$h_i^2 = \frac{2.5\sigma_{f_i}^2}{\sigma_{f_i}^2 + \sigma_{b_i}^2 + \sigma_{e_i}^2} \quad (12)$$

The genetic correlations ( $r_{g_{ij}}$ ) between trait  $i$  and trait  $j$  were calculated as:

$$r_{g_{ij}} = \frac{\sigma_{f_{ij}}}{\sqrt{\sigma_{f_i}^2 + \sigma_{f_j}^2}} \quad (13)$$

and phenotypic correlation ( $r_{p_{ij}}$ ):

$$r_{p_{ij}} = \frac{\sigma_{f_{ij}} + \sigma_{e_{ij}}}{\sqrt{(\sigma_{f_i}^2 + \sigma_{e_i}^2)(\sigma_{f_j}^2 + \sigma_{e_j}^2)}} \quad (14)$$

where  $\sigma_{f_{ij}}$  is the estimated family covariance between trait  $i$  and trait  $j$ ;  $\sigma_{f_i}^2$  and  $\sigma_{f_j}^2$  is the estimated family variance for trait  $i$  and trait  $j$ . The realised genetic gain ( $\Delta G_R$ ) was computed by subtracting the mean breeding values of selected top ratio wood traits from the total mean of the wood trait.

R software [23] was used for all the statistical analysis. The package “lme4” [24] in R was used for all genetic models and “ggplot2” [25] was used for figures plot.

### 3. Results

#### 3.1. Differences of Family in Growth and Wood Properties

The value of WD, DBH, lignin, and HC varied from 0.30 to 0.89 g/cm<sup>-3</sup>, 9.51 to 36.01 cm, 26.61% to 30.90%, and 62.64% to 72.87% respectively. In terms of standard deviation (SD) and coefficient of variation (CV), apparently, DBH had the highest variation than other properties, followed by WD, lignin, and HC phenotypically. The coefficients of variation of DBH and WD reached 27.75% and 23.94% respectively, nearly 10 times larger than that of lignin and holocellulose. Genetically, all four of these wood properties had a reasonably low heritability. Lignin showed a particularly moderate heritability with a value reaching 0.32, followed by DBH, WD, and HC with a heritability of 0.27, 0.24, and 0.18 respectively (Table 1).

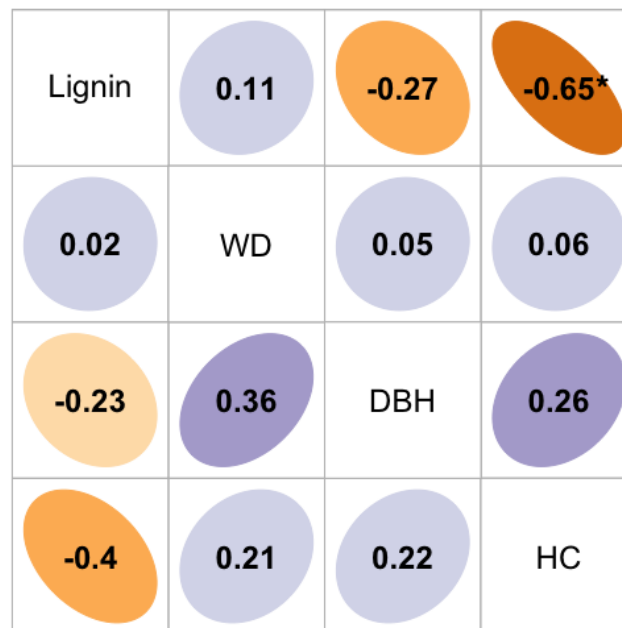
**Table 1.** Descriptive statistics of slash pine wood properties and DBH of 24-year-old *P. elliotii*.

Traits	Max	Mean	Min	SD	CV (%)	$h^2$
WD (g/cm <sup>-3</sup> )	0.89	0.56	0.30	0.15	23.94	0.24
DBH (cm)	36.01	18.15	9.51	7.37	27.75	0.27
Lignin (%)	30.90	28.56	26.61	0.92	3.12	0.32
HC (%)	72.87	67.21	62.24	2.08	2.98	0.18

Note: DBH: Diameter at breast height, WD: Wood density, HC: Holocellulose content,  $h^2$ : Narrow sense heritability.

### 3.2. Genetic and Phenotypic Correlation between Four Wood Properties

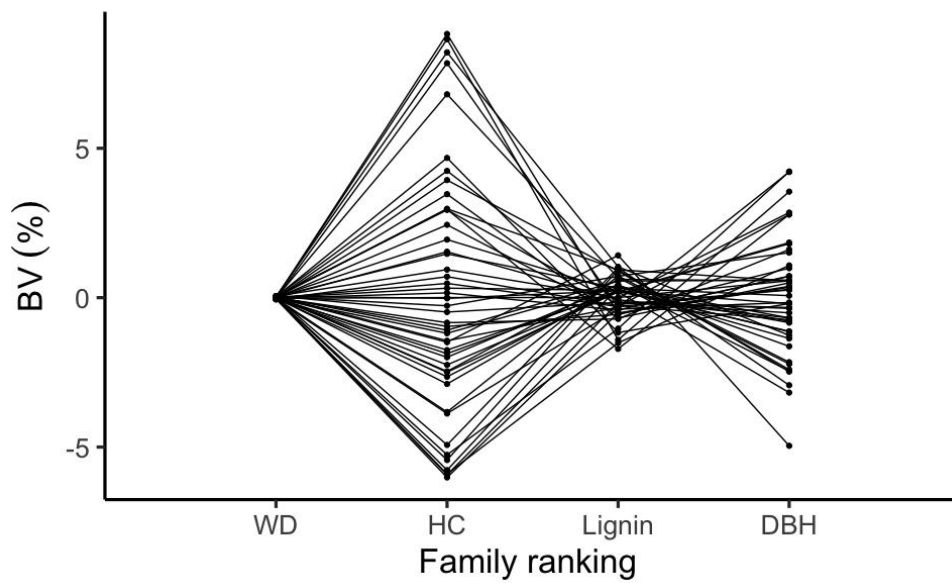
The genetic ( $r_g$ ) and phenotypic correlation ( $r_p$ ) analysis of the four wood property traits of slash pines are displayed in Figure 1. A high and significantly negative genetic correlation ( $r_g = -0.65^*$ ) and a lower phenotypic correlation ( $r_p = -0.4$ ) between lignin and HC were found. WD and DBH were found to have a moderate phenotypic correlation ( $r_p = 0.36$ ) between each other with no significant genetic correlation. WD did not show any correlation with lignin and holocellulose.



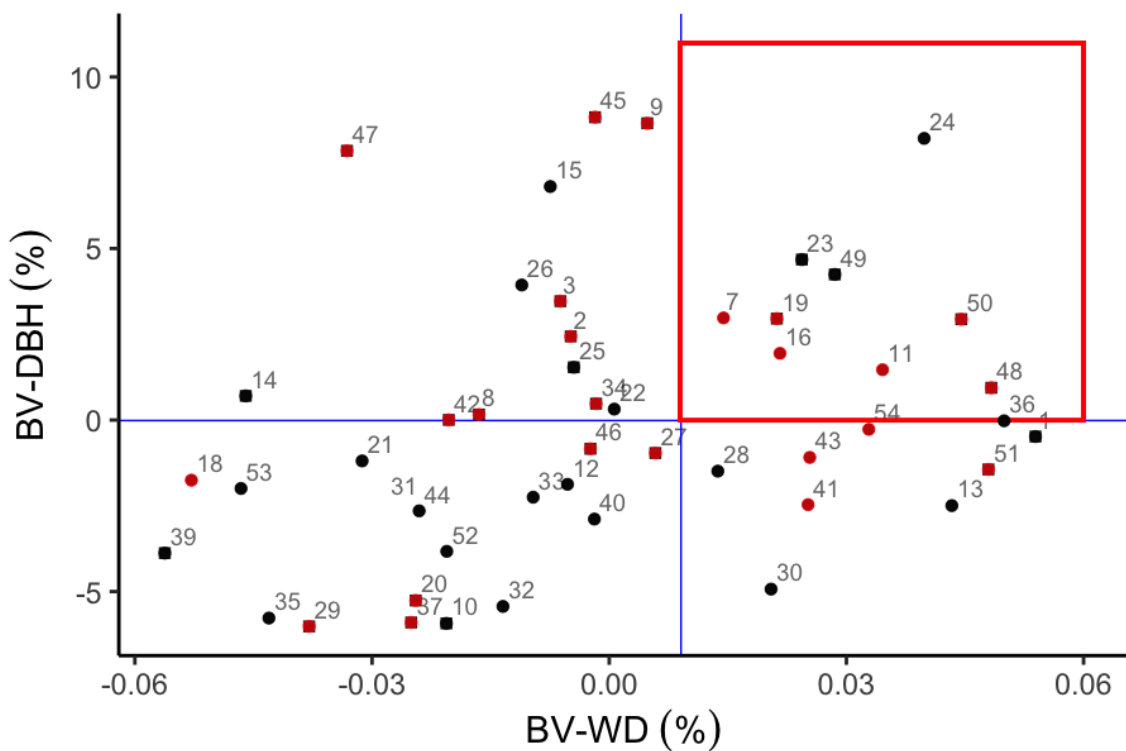
**Figure 1.** Estimates of genetic correlation (up Triangle) and phenotypic correlation (bottom Triangle). DBH: Diameter at breast height, WD: Wood density, HC: Holocellulose content;  $p$ -values significant level: \*: 0.05.

### 3.3. Family Selection

In Figure 2, the breeding values (BV) of WD, DBH, lignin, and HC traits family ranking were plotted. WD had a significantly consistent family ranking with holocellulose. DBH and lignin did not show a highly consistent family ranking as WD and holocellulose. However, it is still possible to select traits by families according to certain purposes. The relationship between WD, DBH, lignin, and HC traits was displayed in Figure 3. The mean breeding values of WD and DBH are displayed in a blue solid line. The breeding values of lignin was higher than the mean value, shown in red and lower than the mean value, shown in black. Similarly, the breeding values of HC were higher than the mean value represented by the dots and lower than the mean value displayed by the square. Different families can be chosen for different purposes of wood properties. For pulp yield, family 24 with a large DBH, high WD and holocellulose, and low lignin content should be considered. In contrast to pulp yield use, family 7, 11, and 16 with large DBH, high WD, lignin, and HC should be the best choice for breeding for industry use. Families in the third quadrant with small DBH and low WD should not be considered for breeding even when high lignin and HC were found.



**Figure 2.** Family rankings for breeding value (BV) of DBH, WD, HC, and lignin in *P. elliottii* at age 24. Each black dot represents one family.

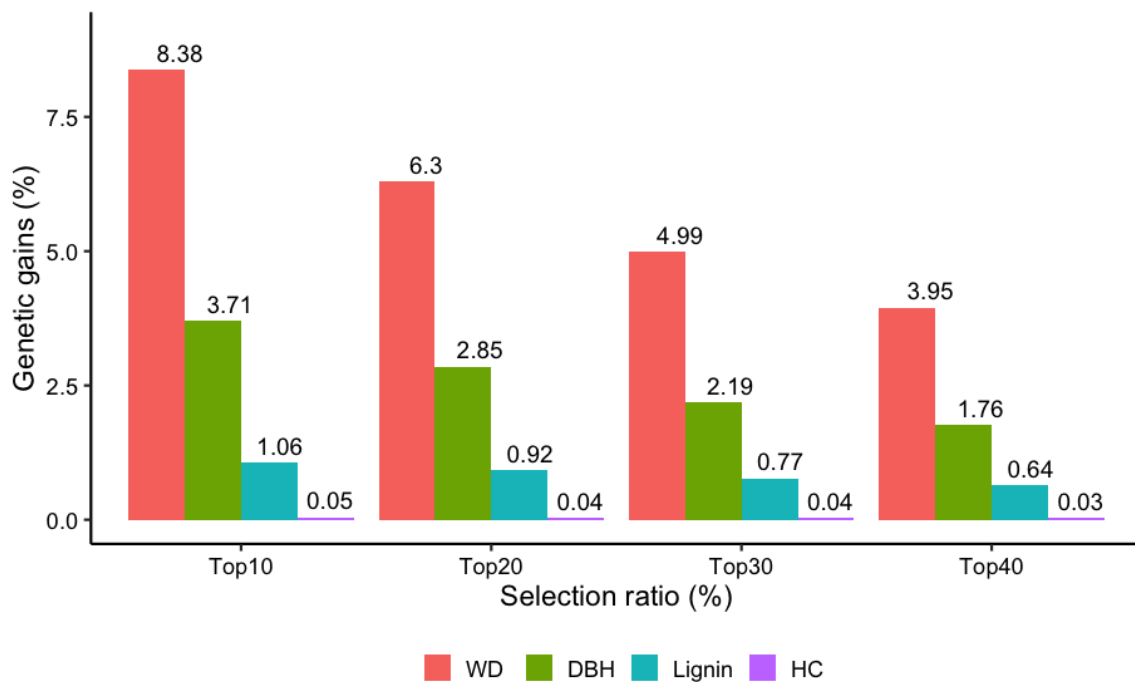


**Figure 3.** Relationship between DBH, WD, HC, and lignin breeding values of *P. elliottii* family. Red and black: Breeding value of lignin above and below mean; dot and square: Breeding value of holocellulose above and below mean; BV-Den: Breeding value of wood density; BV-DBH: Breeding value of diameter at breast height (DBH).

### 3.4. Realised Genetic Gains

The genetics gains were calculated by breeding values with the top 10% to 40% samples from 49 families for each trait. The genetic gain value was shown as an improved percentage, and the results are shown in Figure 4. In general, the top 10% families could produce the highest yield of wood

properties among other selection ratios. The top 10% yielded nearly twice as much as the top 40% selection ratio in WD, DBH, and lignin, but not significantly in holocellulose.



**Figure 4.** Realised genetic gains of four wood traits at age 24 for *P. elliotii*. Selection ratio ranged from 10% to 40% of the total family numbers; genetic gains was calculated as improved percentage.

The largest genetic gain was observed in WD, where the top 10% families could yield 8.38% which was twice as much as the top 40% families (3.95%), followed by DBH, lignin, and holocellulose, with the estimated genetic gains ranged from 3.71% to 1.76%, 1.06% to 0.64%, and 0.05% to 0.03%, respectively.

## 4. Discussion

### 4.1. Genetic Variation Parameters

In our study, the mean of WD was  $0.56 \text{ g/cm}^{-3}$ , which is similar to the mean density that was found in slash pine and Douglas-fir tree [26–28]. Two (WD and DBH) of the four wood traits had a larger CV than the other two (lignin and holocellulose) traits, indicating that there was potentially a genetic variation with *P. elliotii*. Heritability is another important parameter that is mainly used for breeding selection. The inbreeding and hybridisation may complexify the genetic structure of slash pine. Therefore, the coefficient of 2.5 was used for the calculation of heritability to avoid deviations from the assembling of half-siblings and inbreeding effects [22]. In our study, both the lignin and the DBH obtained a moderate heritability with  $h^2$  being 0.32 and 0.27 respectively. This is consistent with the study of Gaspar et al. [29] on DBH and Gaspar et al. [30] on lignin content using 552 17-year-old trees from 46 open-pollinated families of Maritime pine (*Pinus pinaster* Ait.). It suggested that lignin and DBH may be under stronger genetic control. Similar results have been found previously in the *Eucalyptus* species as well [31].

Surprisingly low heritability was found in WD ( $h^2 = 0.24$ ) and HC ( $h^2 = 0.18$ ), which is lower than the results found by Wu et al. [32] who studied the wood density of 180 trees from 30 open-pollinated families in radiata pine and found a heritability of 0.8 at two different sites and Wang et al. [33] who studied the HC on 42 half-sib families in Korean pine (*Pinus koraiensis*) and found a heritability of 0.84. Similar WD heritability was found in Scots pine [34,35] and Norway spruce (*Picea abies*) [36,37] but was lower than that of the Loblolly pine (*Pinus taeda* L.) [38,39]. While heritability is a statistical



concept that describes how much of the variation in a given trait and can be attributed to genetic variation. An estimate of the heritability of a trait is specific to one population and one species in one environment, and it can change over time as circumstances change. The heritabilities estimated in this research would be especially useful to this sampled population of slash pine and had some reference value for other studies.

#### 4.2. Correlations

Understanding the phenotypic and genetic correlations between wood traits is crucial for the tree breeding program [40]. The phenotypic correlations between traits mainly indicate the environmental conditions and the genetic correlations indicate the determination of trait selection. Significantly high negative genetic correlation ( $-0.65$ ) and lower phenotypic correlations ( $-0.4$ ) between lignin and HC were found in our study, which is consistent with the phenotypic and genetic correlations reported in *P. koraiensis* [33]. A high HC associated with low lignin content at both phenotypic and genetic levels are expected. It is indicated that the selection of wood for pulp yield or HC could result in a reduction in lignin content. The relationship between lignin and wood density should be considered, as the wood density is one of the most important wood traits in the slash pine breeding program. WD did not show a strong correlation with DBH, lignin, and HC, with a lower positive genetic correlation at 0.05, 0.11, and 0.06 respectively. This is consistent with the finding by Poke et al. [41] who analysed the genetic parameters of 177 trees from 37 Tasmanian blue gum (*E. globulus*) families. However, a slightly negative genetic correlation was found between density and lignin content in *P. pinaster* [42] which is in contrast to our results. The selection of high basic density with favourable lignin profiles is likely to be possible.

#### 4.3. Family Selection and Genetic Gain

The growth and wood chemical traits combined for selection should be adopted in a long-term breeding program. All of the WD, lignin, and DBH are under strong family genetic control [18]. The same results were also reflected in our study (Table 1). Figure 2 showed that the breeding values of these four wood traits were reasonably consistent among different families. According to this, families with both a high WD, DBH, HC, and a low lignin breeding value were selected as the excellent family for pulp yield production. Similar selections were reported by Li et al. [22] who successfully selected five coast grey box (*Eucalyptus bosistoana*) families with high breeding values of heartwood diameter and heartwood extractive content for tree breeding using this method and then applied it to selecting the high genetic variation of leaf chlorophyll content and colour on Cha mu (*Sassafras tzumu*) [43], which showed that this method is feasible in selecting excellent families.

Genetic gain is often used as a measurement of increasing performance after direct or indirect selection. As for the genetic gain in this study, apparently, a strong genetic selection ratio (10%) could yield better genetic gain in WD and DBH compared to a low selection ratio (40%) and the conclusion is in line with other researches on slash pine and eucalyptus [22,26].

### 5. Conclusions

Wood properties are the most important traits in trees especially for industrial use. Certain traits which are mostly required by the industry mainly determine the important economic application value of wood. In this study, a total number of 1059 individual slash pine trees from 49 families were used for a genetic study of wood properties in slash pine. These estimates of genetic parameters were obtained for the breeding selection analysis. Relatively moderate heritability was found in Lignin ( $h^2 = 0.32$ ), followed by DBH, WD, and HC with a heritability of 0.27, 0.24, and 0.18 respectively. A significant high negative genetic correlation was found between lignin and HC ( $r_g = -0.65^*$ ). WD yielded the highest genetic gains over other properties within the sample of only the top 10% of total families. The WD could be improved 8.38%, followed by the DBH, lignin, and holocellulose, which could be improved 3.71%, 1.06%, and 0.05% respectively. The reasonable consistence of breeding value in DBH, WD,



lignin, and HC around different families indicates that it has the potential to improve wood properties for paper yield through the proposal of a breeding selection that ensures trees with good quality.

**Author Contributions:** Y.L. designed the study, conceived, designed, and performed experiment, analysis the data and drafted the manuscript. X.D. performed lab experiments, J.J. and Q.L. supervised the experiments at all stages and provided critical feedbacks. All authors have read and approved the final manuscript.

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