

Article

Trait Assessment of 1122 *Populus deltoides* Clones: Unveiling Correlations among Growth, Wood Properties, and Disease Susceptibility

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Abstract: This study aimed to evaluate the growth, wood properties, disease susceptibility, and sex traits of 1122 *Populus deltoides* clones to reveal the trait variability and correlations, providing a basis for genetic improvement and breeding. The measurements included the diameter at breast height (DBH), leaf area, basic wood density (BWD), content of cellulose, hemicellulose, lignin, and disease susceptibility index (DSI). The coefficients of variation ranged 6.91%–41.96%, with the BWD showing the lowest variability. Significant sexual dimorphism was observed, with male clones exhibiting higher DBH and hemicellulose content, and female clones displaying larger leaf areas and greater phenotypic variability. Correlation analysis revealed that the leaf area was positively correlated with the BWD and hemicellulose, and it was negatively correlated with the DBH and lignin; lignin was negatively correlated with cellulose. PCA confirmed these relationships and additionally highlighted a positive correlation between the DSI and DBH. These findings established links between the growth traits and wood properties, enhancing our understanding of trait diversity in *P. deltoides* and providing insights for breeding strategies to develop high-quality, high-yielding cultivars.

Keywords: *Populus deltoides*; trait assessment; trait diversity; trait correlation; sexual dimorphism



Citation: Ma, T.; Hou, J. Trait Assessment of 1122 *Populus deltoides* Clones: Unveiling Correlations among Growth, Wood Properties, and Disease Susceptibility. *Forests* **2024**, *15*, 1250. <https://doi.org/10.3390/f15071250>

Received: 22 May 2024

Revised: 29 June 2024

Accepted: 16 July 2024

Published: 18 July 2024



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1. Introduction

Global forests cover 4.06 billion hectares, accounting for 31% of the Earth's land area, making them the most extensive terrestrial ecosystems on the planet [1]. They nurture over 80% of the terrestrial biodiversity [2]. These ecosystems not only provide essential habitats for countless flora and fauna but also supply valuable resources such as timber, medicinal plants, and food for human societies. Forests play a crucial role in maintaining biodiversity, storing carbon, regulating climate, protecting water sources, and improving air quality. Thus, protecting and scientifically managing forest resources is of utmost importance for maintaining the ecological balance regionally and globally, and for promoting sustainable development.

Populus spp., belonging to the genus *Populus* within the family Salicaceae, are renowned for their rapid growth, ease of propagation, early maturity, high yield, and strong adaptability [3,4]. Natural populations of poplar are distributed across several high-latitude regions in China. However, in the warmer temperate and subtropical plains of lower latitudes, there is still a lack of fast-growing, high-quality, and disease-resistant poplar resources. The eastern cottonwood (*P. deltoides*), belonging to Sect. *Aigeiros*, is primarily found in the central and eastern parts of North America, spreading across 31 states in the USA and five provinces in Canada. Its distribution ranges from 28° N to 56° N latitude, spanning north to south over 1900 km and east to west over 2250 km, demonstrating a wide geographical distribution and rich genetic diversity [5]. Based on the climatic conditions of its native habitat, eastern cottonwood can

be categorized into the northern, central, and southern geographical sources. The southern source of eastern cottonwood, notable for its exceptional adaptability, has gained significant attention worldwide for its ability to thrive in the Southern Hemisphere and low-latitude regions. Since 1972, eastern cottonwood has been introduced into China, setting new records for rapid growth and high yield in the middle and lower reaches of the Yangtze River and the Jianghuai Plain [6,7].

Poplar breeding has been highly valued globally. As early as 1912, British scholar Henry John Elwes initiated the world's first artificial hybridization experiment with poplar trees. Through this experiment, he successfully cultivated two asexual varieties that combined the traits of both parents, showing a more significant hybrid vigor compared to natural hybrids [8]. Since then, the artificial hybridization and selection of hybrid clonal lines have become a widely adopted breeding procedure among poplar breeders worldwide. The traits of the parents have a decisive impact on the performance of the hybrids. Effective parental selection is the starting point for the hybrid breeding process, and a detailed phenotypic trait survey is not only the premise and basis for parental selection decisions but also the key to identifying and cultivating new poplar varieties with high yield, excellent quality, and strong stress resistance. Combining phenotypic data with molecular biology and genomics research can help identify genes and molecular markers associated with important traits, accelerating the molecular breeding process and improving the breeding efficiency. By analyzing the correlation between phenotype and genotype, selective breeding can be conducted more precisely, resulting in new poplar varieties with specific desirable traits. Therefore, in-depth phenotypic measurement is essential for successful breeding work.

The growth traits, wood properties, and resistance traits are essential factors determining the quality of poplar trees. The growth traits, including the diameter at breast height (DBH), tree height, and leaf area, directly impact the productivity and economic benefits of the trees. The wood properties, such as the basic wood density, cellulose content, and lignin content, determine the quality and usage of the wood. The resistance traits involve the tree's ability to withstand pests, diseases, and environmental stresses, directly affecting the tree's survival and health [9]. Studies indicate that *P. deltoides* is primarily affected by diseases such as leaf blight, canker, rust, black spot, and leaf spot [10–14]. Additionally, it is vulnerable to wood-boring insects like *Saperda carcharias* and *Platypus mutatus* Chapuis, as well as leaf-feeding insects such as *Clostera*, *Paranthrene tabaniformis* Rott., and the cottonwood leaf beetle [15–19]. The ideal poplar should exhibit rapid growth and strong resistance to increase the wood yield. It should also have moderate basic density and strength, a high cellulose content to support the paper and textile industries, a moderate lignin content to reduce the bleaching costs and improve the biorefining efficiency, and an appropriate hemicellulose content to enhance the physical properties of paper and improve the biomass energy production efficiency [20–22]. *P. deltoides* is a dioecious species [23]. Previous studies have shown that male trees outperform female trees in terms of the DBH, but there are no significant gender differences in the wood density [23,24]. Sexual dimorphism analysis can reveal differences between male and female trees in terms of the growth rate, disease resistance, and wood properties. Incorporating gender differences into breeding evaluation standards can improve the breeding efficiency and optimize the target traits, leading to the cultivation of higher-quality and higher-yielding poplar varieties. Therefore, sex surveys and sexual dimorphism analyses are crucial in poplar breeding research.

The growth traits can be quickly obtained through conventional field measurement tools and techniques, whereas the wood properties typically require laboratory analysis to determine them, including sample collection, sample preparation, chemical analysis, and the use of specialized instruments, which not only increases the costs but also prolongs the experimental period. Therefore, in the breeding and cultivation process, priority is often assigned to selecting varieties with excellent growth traits. However, to ensure that the wood properties are effectively improved, establishing the correlation between the growth traits and wood quality is crucial in wood breeding. These traits are usually complex

quantitative traits controlled by multiple genes [25,26]. When two or more phenotypic traits show correlation, these traits may represent key characteristics of trait variation. By identifying these characteristics and more accurately grasping the genetic correlation between the target traits, the breeding efficiency can be significantly improved [27]. Zhang et al. studied the correlation between the growth traits and wood properties of triploid hybrid clones of *Populus tomentosa* under different environmental conditions and found a significant correlation between the growth traits and wood properties [28]. These findings suggest that by selecting genotypes with excellent growth traits, the wood quality can also be simultaneously improved.

In 1998, the breeding team at Nanjing Forestry University established a germplasm resource bank of *P. deltoides* at the Chenwei Forest Farm in Sihong County, Suqian City, Jiangsu Province. To compare the trait differences among the 1122 *P. deltoides* clones collected in the germplasm resource bank, one-year-old shoots from these 1122 clones were cut and propagated by cuttings at the Malanghu Branch of the Chenwei Forest Farm in the spring of 2014. Currently, these plants have reached maturity, making them suitable for parental selection and the breeding of superior poplar varieties. Based on this, a comprehensive evaluation was conducted of the growth traits, wood properties, disease resistance, and sex traits of these 1122 *P. deltoides* clones. Through correlation analysis and principal component analysis, the variability and correlations among these traits were revealed, providing scientific evidence for future genetic improvement and breeding work, and laying the foundation for further elucidating the genetic characteristics of key traits in *P. deltoides*.

2. Materials and Methods

2.1. Description of the Experimental Site

The experimental site is located at Malang Lake Subfield, Chenwei Forest Farm, Sihong County, Suqian City, Jiangsu Province, China (33°05' N, 118°18' E). This region falls under the East Asian monsoon climate and serves as a transitional zone between the subtropical and warm temperate zones. The area experiences distinct monsoon seasons and clear seasonal changes. The annual average temperature is 14.6 °C, with rainfall being relatively concentrated, leading to an annual average precipitation of 893.9 mm. The soil type is sandy loam, characterized by high fertility, making it highly suitable for the growth of *Populus deltoides* [29,30].

2.2. Experimental Materials

In the spring of 2014, the breeding team at Nanjing Forestry University took cuttings from one-year-old shoots of 1122 clones collected from the *P. deltoides* germplasm resource bank and propagated them at the Malanghu Branch of the Chenwei Forest Farm. After successful rooting, these clones have been growing naturally at the Malanghu Branch of the Chenwei Forest Farm without human intervention. In 2023, we conducted a trait survey of these clones, which had been growing for nearly 10 years since the cuttings were propagated.

2.3. Trait Measurement

2.3.1. Growth Trait Measurement

The growth traits include the diameter at breast height (DBH) and leaf area of the eastern cottonwood clones. The DBH of each clone was measured at a height of 1.3 m above the ground using a diameter tape, and the data were recorded. From the lower part (at a height of 10–15 m above the ground) of each clone, 10 relatively intact leaves were randomly selected, and their images were captured using a camera. Subsequently, the images were processed with ImageJ software (V1.54) [31] to calculate the leaf area.

2.3.2. Wood Property Measurement

Basic Wood Density Measurement

At a height of 1.3 m above the ground, wood cores were extracted perpendicularly to the trunk using a Haglöf increment borer (5 mm. Haglöf Sweden AB, Långsele, Sweden) [32,33]. The extracted wood cores were stored in numbered custom plastic tubes (300 mm × 8 mm × 9 mm). The basic wood density (BWD) was measured following the standard method outlined in “Testing basic wood density of national dominant species (group)” [34]. The plastic tubes containing the wood cores were filled with distilled water, which was changed daily for 7 days. The wood cores were weighed daily, with the surface water being blotted off using absorbent paper before each weighing. This process continued until the mass of the wood cores remained constant or changed very little, indicating that the wood cores had reached their saturation moisture content. The volume of each wood core at the saturation moisture content ($V_{\max}$ (cm³)) was then measured. Subsequently, the wood cores were dried in an oven at 103 ± 2 °C for 8 h. After removal from the oven, they were placed in a desiccator for 15 min. Five to six samples were selected and weighed using a precision electronic balance accurate to 1 mg. This weighing process was repeated every 2 h until the difference between two consecutive weighing results was less than 0.5% of the sample’s mass, indicating that the samples had reached an oven-dry state. The recorded mass at this point was the oven-dry mass (m (g)). The basic wood density was then calculated using the formula: $\rho = m/V_{\max}$ (g/cm³).

Measurement of Cellulose, Hemicellulose, and Lignin Content

The samples used for the basic wood density measurement were ground into powder using a sample grinder (28,000 r/min, Shanghai Lingsum Technology Co., Ltd., Shanghai, China), with each sample being ground twice (3 min each time). The resulting powder was then sieved multiple times through a 40-mesh sieve, and the sieved powder was stored in numbered Ziplock bags. The Van Soest fiber analysis method was employed to measure the content of cellulose, hemicellulose, and lignin using an F12A automatic fiber analyzer (Shanghai Shengsheng Automation Instrument Co., Ltd., Shanghai, China) [35]. A 0.5 g sample of the sieved powder was weighed using a balance and placed into a numbered filter bag, which was then sealed using a heat sealer. A hole was punched at the top edge of the filter bag using a hole puncher. The filter bags containing the samples were weighed using an electronic balance, and the mass was recorded. The filter bags were then placed in the F12A automatic fiber analyzer (which can process 30 samples at a time). The samples were boiled in a neutral detergent fiber solution for 75 min, rinsed three times with distilled water (8 min each time), and air-dried. They were then soaked in acetone for 30 min and dried to a constant weight in an oven at 105 °C. The remaining residue, known as neutral detergent fiber (NDF), consists mainly of cell wall components such as hemicellulose, cellulose, lignin, and silicates. The dissolved portion is referred to as neutral detergent solubles (NDSs), which include components like fats, sugars, starches, and proteins. Similarly, a 0.5 g sample of the sieved powder was treated with an acid detergent fiber solution for 60 min, rinsed three times with distilled water (8 min each time), soaked in acetone for 30 min, and dried to a constant weight in an oven at 105 °C. The resulting residue, known as acid detergent fiber (ADF), includes cellulose, lignin, and silicates. The ADF was then treated with 72% sulfuric acid for 240 min, rinsed with distilled water, and dried to a constant weight in an oven at 105 °C to obtain acid detergent lignin (ADL). The ADL was ashed in a muffle furnace at 550 °C for 240 min. After cooling to 200 °C, the samples were placed in a desiccator for 30 min and then weighed to obtain acid-insoluble ash (AIA). The content of cellulose, hemicellulose, and lignin in the samples was calculated using the following formulas:

$$\text{Cellulose content} = \text{ADF (\%)} - \text{ADL (\%)}$$

$$\text{Hemicellulose content} = \text{NDF (\%)} - \text{ADF (\%)}$$

$$\text{Lignin content} = \text{ADL (\%)} - \text{AIA (\%)}$$

2.3.3. Determination of Disease Susceptibility Traits

Ten relatively intact leaves from the lower part (at a height of 10–15 m above the ground) of each clone were randomly selected, and photographs were taken using a camera to capture images of these leaves. The images were then processed using ImageJ software [31], employing the Trainable Weka Segmentation plugin to analyze and calculate the percentage of leaf area affected by lesions. The susceptibility of each clone was graded according to the criteria outlined in Table 1 [36]. The disease susceptibility index (DSI) for each clone was calculated using the following formula:

$$\text{Disease susceptibility Index} = \frac{\sum(\text{Representative Value of Each Damage Level} \times \text{Number of Leaves at That Level}) \times 100}{\text{Representative Value of the Highest Damage Level} \times \text{Total Number of Sampled Leaves}}$$

Table 1. Disease grading standards for *Populus deltoides* clones.

Lesion Coverage Percentage	Grade	Representative Value
0%	I	0
0%–25%	II	1
25%–50%	III	2
50%–75%	IV	3
75%–100%	V	4

2.3.4. Determination of Sexual Traits

In April 2023, each clone was observed using a telescope, and the sex of the plant was determined based on the morphology of the catkins. Male catkins open slightly earlier than female catkins. When they first elongate, they are light red. As the catkins mature, their length increases, and the color changes to bright red. Mature catkins can reach a length of about 10 cm. Under full sunlight or at least 6 h of direct sunlight per day, and with temperatures typically ranging from 15 to 25 °C, the anthers split open and pale yellow or light-yellow pollen is dispersed. Female catkins are light yellow–green from the time they start to elongate until just before pollination. When mature, the catkins are 10–12 cm long and contain 18–25 small flowers. After pollination, the stigmas gradually wither and the ovaries begin to swell. Once the seeds are mature, the seed pods split open, releasing white fluff.

2.4. Data Analysis

The measurement data concerning the growth traits, wood properties, disease susceptibility traits, and sex traits of 1122 *P. deltoides* clones were recorded using Microsoft Excel (Redmond, WA, USA). Descriptive statistics, principal component analysis (PCA), and independent sample *t*-tests were performed using SPSS (V25.0) to summarize and describe the basic features of the data, determine the traits that contribute the most to the data variability, and analyze whether there are significant statistical differences in specific phenotypic traits between male and female plants, respectively. Hierarchical clustering analysis and plotting of the disease susceptibility traits were conducted using the factoextra, cluster, and ggplot2 packages in R (V4.2.2) to reveal the relationships and patterns of disease susceptibility among the different clones. The qqplotr package was used to create QQ plots for each trait, to check whether the data distribution conformed to a normal distribution, using the shapiro.test function from the base stats package. Spearman correlation analysis was performed using the Hmisc and corrplot packages to assess the correlations between traits.

3. Results

3.1. Analysis of Growth Traits

Measurements of the diameter at breast height (DBH) and leaf area of the 1122 clones of *P. deltoides* showed that the minimum DBH was 12.30 cm and the maximum was 37.50 cm,

with an average of $28.11 \text{ cm} \pm 0.18 \text{ cm}$ and a coefficient of variation of 11.81%. The leaf area measurements averaged $81.36 \text{ cm}^2 \pm 0.93 \text{ cm}^2$, ranging from 33.36 cm^2 to 159.70 cm^2 , with a coefficient of variation of 27.52%. The Shapiro–Wilk test results for the DBH and leaf area indicate significant deviations from normality. For the DBH, the Shapiro–Wilk statistic was 0.987, with a p -value of 0.008 (Supplementary Table S2), suggesting a significant deviation from normality. The QQ plot for the DBH shows that while the data points align well with the reference line in the middle, there are noticeable deviations at the extremes (Figure 1a), indicating some departure from a normal distribution. For the leaf area, the Shapiro–Wilk statistic was 0.976, with a p -value of 0.000 (Supplementary Table S2), also indicating a significant deviation from normality. The QQ plot for the leaf area shows that the data points are mostly near the reference line but deviate significantly at the higher end (Figure 1b), indicating a departure from normality, especially in the upper range of the data. These findings highlight that both the DBH and leaf area data exhibit significant departures from normality, particularly at the extremes and upper ends, respectively.

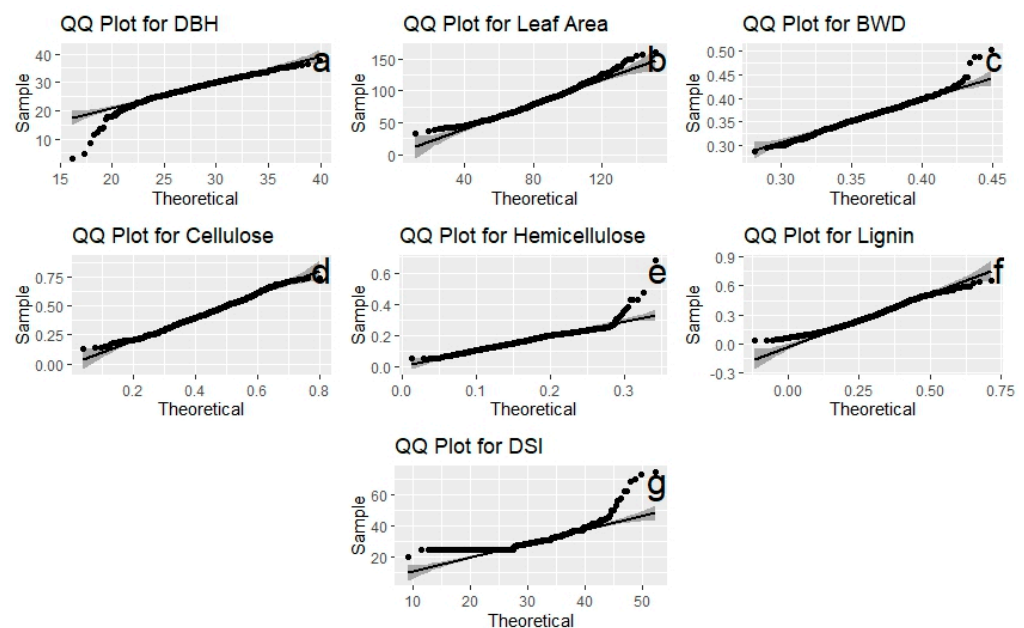


Figure 1. (a) QQ plot for the DBH; (b) QQ plot for the leaf area; (c) QQ plot for the BWD; (d) QQ plot for cellulose; (e) QQ plot for hemicellulose; (f) QQ plot for lignin; and (g) QQ plot for the disease susceptibility index.

3.2. Analysis of Wood Properties

Using the wood core samples extracted with a Haglof increment borer, with a diameter of 0.5 cm and a length of 20 cm, an analysis was conducted on the basic wood density and chemical composition (cellulose, hemicellulose, lignin) of the 1122 clones of *Populus deltoides*. The results revealed the following. The average basic wood density of the population was $0.365 \text{ g/cm}^3 \pm 0.001 \text{ g/cm}^3$, with a range from 0.287 g/cm^3 to 0.504 g/cm^3 and a coefficient of variation of 6.91%. The average cellulose content was $42.03\% \pm 0.36\%$, ranging from 13.30% to 73.52% and with a coefficient of variation of 27.53%. The average hemicellulose content was $17.69\% \pm 0.16\%$, fluctuating between 5.20% and 67.80%, with a coefficient of variation of 28.32%. The average lignin content was $29.93\% \pm 0.39\%$, with a range between 2.85% and 65.78% and a coefficient of variation of 41.96%. The Shapiro–Wilk test results for the BWD, cellulose, hemicellulose, and lignin indicate varying degrees of normality. The BWD and lignin both had significant deviations from normality, with p -values of 0.000 (Supplementary Table S2). Their QQ plots showed some alignment with the reference line but deviated at the tails (Figure 1c,f). Hemicellulose also significantly deviated from normality (p -value of 0.000) (Supplementary Table S2), with the QQ plot showing notable deviations at the higher end (Figure 1e). In contrast, cellulose did not

significantly deviate from normality (p -value of 0.147) (Supplementary Table S2), and its QQ plot closely followed the reference line (Figure 1d). These findings highlight that while the cellulose data approximate a normal distribution, the BWD, hemicellulose, and lignin data exhibit significant departures from normality, indicating substantial variability in these traits among the *P. deltoides* clones.

3.3. Analysis of Disease Susceptibility Traits

A survey of the disease incidence among the 1122 clones of *P. deltoides* revealed that the primary disease type is black spot (Figure 2), with a few individuals also infected by both black spot and rust. The pathogen causing leaf spot in *P. deltoides* is *Marssonina brunnea* [37], while the pathogen causing rust is *Melampsora laricis-populina* [38]. Leaf spot causes black–brown lesions on the leaves, whereas rust produces yellow pustules. Due to the very low incidence of rust, this study focused solely on the susceptibility of *P. deltoides* clones to leaf spot. By calculating and analyzing the DSI of these clones, the results show that the minimum DSI is 20% and the maximum is 75%, with an average of $30.68\% \pm 0.29\%$ and a coefficient of variation of 22.40%. These results demonstrate the wide distribution and significant diversity of the DSI within the population. To further understand the population distribution characteristics and structure of the DSI, the Shapiro–Wilk test was conducted. It indicates a significant deviation from normality, with a Shapiro–Wilk statistic of 0.767 and a p -value of 0.000 (Supplementary Table S2). This suggests that the DSI data do not follow a normal distribution. The QQ plot corroborates this finding, showing substantial deviations from the reference line at both the lower and upper ends of the distribution (Figure 1g). These results highlight that the DSI data significantly depart from a normal distribution, indicating considerable variability among the *P. deltoides* clones in terms of the disease susceptibility.

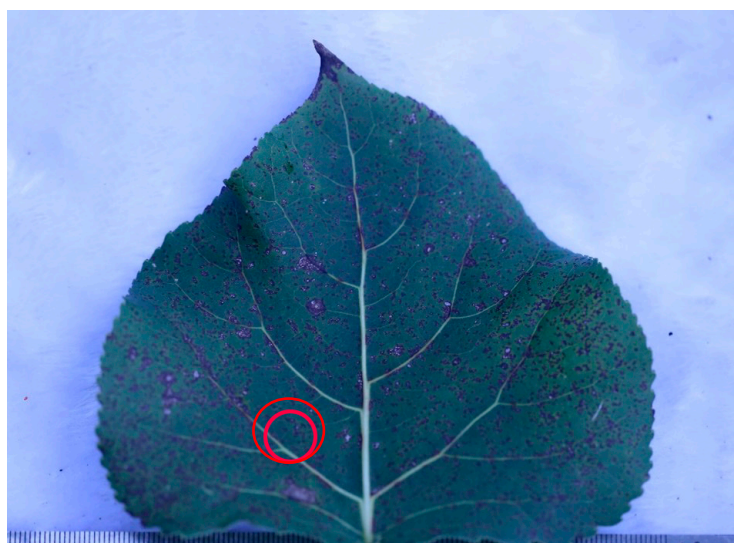


Figure 2. *Populus deltoides* leaves infected with black spot. Note: the red circles are black spot lesions.

The cluster analysis shown in Figure 3 effectively divides the 1122 *P. deltoides* clones into five distinct groups, with relatively consistent DSI values within each group. The specific distribution is as follows: Group 5 (56.25%–75%) contains 9 highly susceptible individuals; Group 4 (38.46%–53.125%) includes 47 individuals; Group 3 (30.56%–37.5%) includes 182 individuals; Group 2 (25%–30%) includes 339 individuals; and Group 1 (less than 25%) contains 545 individuals with strong disease resistance. The DSI shows a decreasing trend from Group 5 to Group 1, reflecting a gradient from susceptibility to resistance. In this population, the number of highly susceptible individuals is relatively small, while the majority of individuals exhibit strong disease resistance.

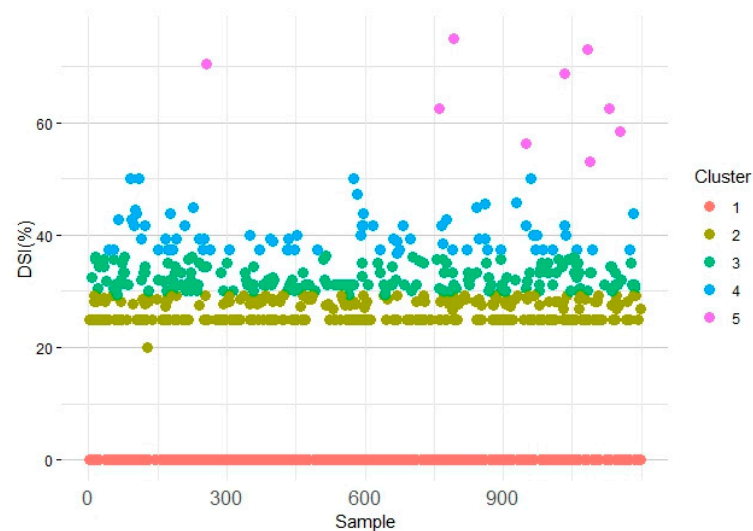


Figure 3. Cluster analysis of the disease susceptibility index in *Populus deltoides* clones.

3.4. Analysis of Sexual Dimorphism

Among the 1122 clones of *P. deltoides*, 836 are female and 286 are male. The statistical analysis of the DBH, leaf area, BWD, contents of cellulose, hemicellulose, and lignin, and DSI categorized by sex showed significant differences between the sexes. The DBH ($p < 0.01$) and hemicellulose content ($p < 0.05$) of male clones were significantly higher than those of female clones, while the leaf area of female clones was significantly larger than that of male clones ($p < 0.01$). In terms of the basic wood density, cellulose content, lignin content, and DSI, female clones were slightly higher than male clones, but the differences were not significant. Among the seven traits, except for the coefficient of variation of the leaf area being slightly higher in male clones, the coefficients of variation for the other six traits were higher in female clones than in male clones, indicating greater trait variability in female clones (Table 2).

Table 2. Phenotypic trait analysis of *Populus deltoides* clones categorized by sex.

Traits	Sex	Mean $\pm$ Standard Errors	Range	Coefficient of Variation (%)
DBH (cm)	Female	27.74 $\pm$ 0.12 b	12.30–37.50	12.09
	Male	29.20 $\pm$ 0.18 a	18.00–35.50	10.16
Leaf area (cm ²)	Female	83.43 $\pm$ 1.06 a	33.36–159.70	26.70
	Male	74.41 $\pm$ 1.86 b	41.41–157.52	28.79
BWD (g/cm ³)	Female	0.366 $\pm$ 0.000 a	0.287–0.504	7.18
	Male	0.363 $\pm$ 0.001 a	0.300–0.487	5.99
Cellulose (%)	Female	42.23 $\pm$ 0.44 a	13.30–73.43	28.47
	Male	41.48 $\pm$ 0.62 a	17.95–73.52	24.81
Hemicellulose (%)	Female	17.49 $\pm$ 0.19 b	5.20–67.80	34.78
	Male	18.26 $\pm$ 0.27 a	5.29–36.50	24.61
Lignin (%)	Female	30.01 $\pm$ 0.47 a	2.84–65.80	43.34
	Male	29.70 $\pm$ 0.69 a	5.39–58.14	37.79
DSI (%)	Female	30.90 $\pm$ 0.34 a	25.00–75.00	22.90
	Male	29.80 $\pm$ 0.49 a	20.00–50.00	18.97

Note: different low case letters indicate statistical differences at $p < 0.05$.

3.5. Trait Correlation Analysis

The Spearman correlation analysis shows that there are more traits significantly correlated with the leaf area and lignin. Specifically, the leaf area is significantly positively correlated with the BWD (correlation coefficient = 0.117, $p < 0.01$) and hemicellulose (correlation coefficient = 0.187, $p < 0.01$), and it is significantly negatively correlated with the DBH (correlation coefficient = -0.084 , $p < 0.05$) and lignin (correlation coefficient = -0.111 , $p < 0.01$).

Lignin is significantly negatively correlated with cellulose (correlation coefficient = -0.814 , $p < 0.01$), hemicellulose (correlation coefficient = -0.350 , $p < 0.01$), and the DSI (correlation coefficient = -0.125 , $p < 0.05$). The DSI is significantly positively correlated with the BWD (correlation coefficient = 0.084 , $p < 0.01$) and hemicellulose (correlation coefficient = 0.224 , $p < 0.01$). Cellulose is only significantly negatively correlated with lignin (correlation coefficient = -0.814 , $p < 0.01$) and has no significant correlation with the other traits (Figure 4).

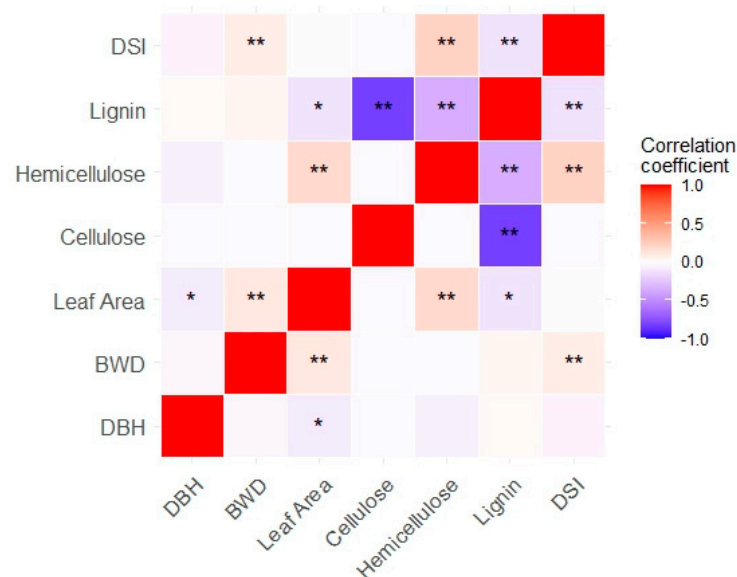


Figure 4. Heatmap of the correlations among seven phenotypic traits in *Populus deltoides* clones. Note: * indicates $p < 0.05$; ** indicates $p < 0.01$.

3.6. Principal Component Analysis

To better understand the relationships among the traits and the main sources of variation, we conducted a PCA. Table 3 presents the loadings and variance contributions of seven phenotypic traits on the first four principal components (PC1, PC2, PC3, PC4). PC1 reveals a significant opposition between cellulose and lignin, with cellulose showing a high positive loading (0.845) and lignin exhibiting a high negative loading (-0.973). This suggests that clones with high cellulose content generally have low lignin content and vice versa; this component explains 26.507% of the total variance. PC2 primarily reflects the positive correlation between the leaf area, BWD, and hemicellulose, with the highest loading for the leaf area (0.723) and considerable loadings for the BWD and hemicellulose (0.518 and 0.493, respectively). The variance contribution rate for PC2 is 16.818%. PC3 highlights the association between the BWD and DBH, with a high loading for the DBH (0.363) and a significant loading for the BWD (0.578), explaining 16.101% of the variance. PC4 reveals significant positive loadings for the DBH (0.816) and DSI (0.499), explaining 14.876% of the variance. These data, revealed through the PCA, highlight the relationships among the traits and the main sources of variation.

Table 3. Results of principal component analysis for phenotypic traits in *Populus deltoides* clones.

Traits	Principal Component			
	PC1	PC2	PC3	PC4
DBH	−0.167	−0.152	0.363	0.816
Leaf area	0.133	0.723	0.141	−0.183
BWD	−0.084	0.518	0.578	0.157
Cellulose	0.845	−0.350	0.248	−0.003

Table 3. Cont.

Traits	Principal Component			
	PC1	PC2	PC3	PC4
Hemicellulose	0.465	0.493	−0.278	0.167
Lignin	−0.973	0.037	−0.094	−0.035
DSI	0.128	0.187	−0.636	0.499
Variance contribution rate (%)	26.507	16.818	16.101	14.876
Cumulative contribution rate (%)	26.507	43.326	59.427	74.303

4. Discussion

This study conducted detailed measurements of 1122 *P. deltoides* clones, assessing their growth traits (DBH, leaf area), wood properties (BWD, content of cellulose, hemicellulose, and lignin), and DSI. The results showed that the coefficients of variation for these traits ranged from 6.91% to 41.96%. Among them, the BWD exhibited the smallest coefficient of variation, less than 10%, indicating relatively low variability; the coefficients of variation for the other six traits were all above 10%, showing higher variability. These findings suggest significant individual differences within the clonal population of *P. deltoides*. Normality tests and QQ plots revealed that, with the exception of cellulose, most traits did not follow a normal distribution. This deviation from normality, particularly observed in the case of the DBH, BWD, leaf area, hemicellulose, lignin, and DSI, suggests non-normal data distribution patterns. Understanding the distribution and variability of these traits is crucial for the breeding program. Traits with low variability, such as the BWD, provide a stable foundation for selection, ensuring consistency in wood quality. The high variability observed in the other traits suggests a broad genetic base, which is beneficial for selecting hybrid parents with desirable combinations of traits. By identifying superior individuals and understanding the distribution patterns, breeders can predict the potential success of hybrids more effectively, thus enhancing the efficiency and effectiveness of breeding programs aimed at improving growth, wood quality, and disease resistance in *P. deltoides*.

Spearman correlation analysis revealed significant correlations among several key traits in the *P. deltoides* clones. For instance, the leaf area was positively correlated with the BWD and hemicellulose content, but negatively correlated with the DBH and lignin content. This indicates that clones with larger leaf areas typically have higher wood density and hemicellulose content but smaller DBH and lower lignin content. Additionally, lignin was negatively correlated with cellulose, hemicellulose, and the DSI, suggesting that clones with higher lignin content generally have lower cellulose and hemicellulose content and lower disease susceptibility. These results are consistent with previous studies, further confirming the interrelationships among these traits [39–41]. PCA revealed several primary sources of variation. PC1 highlighted a significant opposition between cellulose and lignin, indicating that in these clones, high cellulose content typically corresponds to low lignin content, and vice versa. PC2 primarily reflected the positive correlations between the leaf area, BWD, and hemicellulose, further confirming the link between the growth traits and wood properties. PC3 and PC4 revealed significant associations between the DBH and BWD, and the DBH and DSI, respectively. These findings have important implications for future breeding programs. Understanding the positive correlations between the leaf area, BWD, and hemicellulose can help breeders select superior clones with larger leaf areas and higher wood density, thereby enhancing the wood quality. Moreover, the opposition between lignin and cellulose indicates that when selecting clones with high cellulose content, it is essential to consider the reduction in lignin content, which is crucial for pulp and biofuel production [41,42]. The positive correlation between the DBH and DSI suggests that clones with larger DBH may be more susceptible to disease, thus requiring a balance between the growth rate and disease resistance in breeding programs.

The sex of plants plays a crucial role in the selection of hybrid parents and the breeding of superior cultivars. By pairing parents of different sexes for crossing, genetic diversity

and heterosis can be increased, and trait complementation can be achieved. Our study focused on the sexual dimorphism in *P. deltoides*, revealing significant differences in several key traits between male and female clones. A survey of 1122 eastern cottonwood clones revealed that the number of female plants was significantly higher than that of male plants. Specifically, male clones exhibited significantly larger DBH and higher hemicellulose content compared to female clones. This advantage may reflect the adaptability of male clones in terms of the growth rate and structural support, consistent with previous studies [43–46], indicating that male plants typically invest more resources in growth and competition to enhance reproductive success [45,46]. In contrast, female clones had significantly larger leaf areas, which likely correlates with their higher photosynthetic capacity. This increased leaf area supports the energy-intensive process of developing reproductive organs such as fruits and seeds, suggesting that female clones allocate more resources to reproduction [47–51]. These findings highlight the importance of considering the leaf area and photosynthetic capacity in breeding programs aimed at improving reproductive traits. Additionally, the wood chemical composition differed between the sexes, with female clones showing slightly higher cellulose content, making them particularly valuable for industries such as paper and textiles [52]. In contrast, male clones with higher hemicellulose content are advantageous for certain biomass energy production processes [20–22]. The slightly higher lignin content in female clones contributes to better mechanical properties [53,54], although the impact of sex on wood density was relatively minor. Regarding the DSI, our analysis indicated a slightly higher DSI in female clones compared to male clones, suggesting that female clones may exhibit increased disease susceptibility. This finding is pivotal for breeding programs focused on enhancing disease resistance, underscoring the necessity of integrating sex-specific traits into the selection criteria. Consistent with our results, previous studies have reported that male plants often demonstrate lower disease susceptibility relative to female plants [55]. By incorporating these observed DSI differences into breeding strategies, breeders can potentially develop more resilient cultivars, leveraging the lower susceptibility and enhanced vigor of male clones. Furthermore, the greater coefficients of variation for various traits in female clones compared to male clones indicate higher phenotypic variability in females. This variability reflects the enhanced phenotypic plasticity of female clones in response to environmental fluctuations, which can be advantageous in breeding programs aiming to improve adaptability and resilience. These findings on sexual dimorphism in terms of growth, wood chemical composition, and disease resistance provide critical insights for optimizing breeding strategies in *P. deltoides*. By understanding and capitalizing on these sex-specific traits, breeders can more precisely select and cultivate new cultivars that align with specific production objectives, ultimately enhancing both the quality and yield.

5. Conclusions

This study evaluated the growth, wood properties, disease susceptibility, and sex traits of 1122 *P. deltoides* clones, revealing significant variability and correlations. Most clones showed strong disease resistance. Additionally, female clones had larger leaf areas, while male clones had higher DBH and hemicellulose content. Correlation analysis revealed that the leaf area was positively correlated with the BWD and hemicellulose, and negatively with the DBH and lignin. Moreover, lignin was negatively correlated with cellulose. PCA confirmed these relationships and additionally highlighted a positive correlation between the DSI and DBH. These findings enhance our understanding of the traits of *P. deltoides*, providing a solid foundation for future breeding efforts aimed at improving the growth, wood quality, and disease resistance.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f15071250/s1>, Table S1: Components of Detergent Fiber Solution.; Table S2: Shapiro-Wilk Test Results for Normality of Various Traits.

Author Contributions: Design, T.M. and J.H.; writing, T.M. and J.H. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Key Research and Development Project of Jiangsu Province, China (BE2021366).

Data Availability Statement: Dataset available on request from the authors. The raw data supporting the conclusions of this article will be made available by the authors on request.

Conflicts of Interest: The authors declare no conflicts of interest.

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