

Radial Variation Studies on Wood Properties of *Populus deltoides* Parents and Their Hybrids

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The radial variation and the genetic variation of wood properties between the parents and offspring of *Populus deltoides* were studied in this work. The chemical composition, density, and anatomical characteristics of *Populus deltoides* cl. 'Danhong' and its offspring exhibited the phenomenon of transgressive segregation. The chemical compositions of the parents and offspring were decreased in several attributes (benzene alcohol extract, hemicelluloses, lignin) with the increase of the cambial age. Moreover, the fibre length, fibre width, ratio of fibre length to width, and wall thickness to lumen ratio of parents and offspring were increased with cambial age. In addition, the densities of parents and offspring were increased with the increase of cambial age. There were significant differences in wood properties among *Populus deltoides* and its hybrids. These results indicated that *Populus deltoides* cl. 'Danhong' could be considered as pulp material and *Populus deltoides* cl. 'Nanyang' as building material. According to the radial variation rule of each material character, the rotation cutting period can be selected as years 7 or 8.

Keywords: *Populus deltoides*; Hybrids; Chemical composition; Density; Fibre morphology; Radial variation

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INTRODUCTION

Due to its fast growth, strong adaptability, and short rotation period, poplar (*Populus*) is one of the most extensively cultivated species and has a high yield in the world's mid-latitude forest regions (Du *et al.* 2013; Jacobsen *et al.* 2018). The planting area of poplar in China has reached over 7 million hm², ranking first in the world. Poplar wood is mainly used as pulp wood and plywood because its cellulose, lignin, fibre length, fibre width, and wood density are ideal pulp properties that are related to the quality of the final paper product. Thus, poplar has an important economic significance in pulp production; however, with respect to wood products, it has always been neglected (Eriksson 1990; Barnett and Bonham 2004; Marques *et al.* 2010; Hamzeh *et al.* 2011; Weng *et al.* 2011; Gandini and Pasquin 2012; Huang *et al.* 2012; Van den Bosch *et al.* 2015).

Crossbreeding is an important approach for poplar breeding. The hybrid offspring have 1.6 to 4.6 times better physical and chemical pulp properties, and they also have improved fibre morphology and yield compared to the pure poplar clones (Marzena *et al.* 2019). The quality of trees and the potential for genetic improvement should be considered when selecting forest farm tree varieties. Research on poplar parents and offspring have mainly focused on genotypes, leaf morphology, and insect pest resistance, while studies on radial variation and the law of genetic variation have been fewer, not comprehensive, and not systematic (Lippman and Zamir 2007; Chen 2010; Adebayo *et al.* 2011; Baranwal *et*

al. 2012; Frankham 2015; Ren *et al.* 2017). Zhang *et al.* (2012) studied the genetic control of growth traits (tree height, diameter at breast height, stem volume) and wood characteristics (basic wood density and fibre length) of triploid hybrid clones of *Populus*, and the results showed that wood traits were more stable than growth traits. The parents and hybrid progenies of *Populus deltoides* have also been studied and accurate indexes of the primary wood properties have been created.

The morphological characteristics of wood, such as cellulose, lignin, density, fiber length, and microfibril angle, are closely related to the properties of pulp and paper (Parham *et al.* 1997; Wu *et al.* 2011). Cellulose is not only the main component of the fiber cell wall, but also is the main component of pulp. In fact, the goal while producing chemical pulp is to remove lignin from plant fiber raw materials, while retaining cellulose and a certain amount of hemicellulose as much as possible (Georgieva *et al.* 2008; Forouzanfar *et al.* 2016). The cellulose content of raw materials directly affects the pulping yield. In addition, the arrangement of cellulose macromolecules in wood raw materials also affects the pulping yield and pulp quality (Peng *et al.* 2014; Sun *et al.* 2014; Osong *et al.* 2016). The longer the fiber length is, the contact sites and area between the pulp fibers also increase accordingly, and the comprehensive physical properties of the paper can be guaranteed (Fardim *et al.* 2012; Wu *et al.* 2014). Fiber width, wall thickness to lumen ratio, and ratio of lumen diameter to wall thickness are the standards of material quality. With the increase of the wall thickness to lumen ratio, paper tensile strength and burst index increase, but the tear index decreases (Stratton *et al.* 1990; Syed *et al.* 2016).

Populus deltoides cl. '55/65' was introduced from the Norwest Poplar Research Institute in Yugoslavia in 1981, and the *Populus deltoides* cl. '2KEN8' from Italy was introduced in 1980. Both are the primary varieties of the industrial timber forest poplar in China (Zhang *et al.* 2008; Hu *et al.* 2013). The varieties (*Populus deltoides* cl. 'Danhong' and *Populus deltoides* cl. 'Nanyang') were selected by artificial pollination with *Populus deltoides* cl. '55/65' and *Populus deltoides* cl. '2KEN8' as parents. The purpose of this study was to detect the radial variation and the law of genetic variation between the parents and offspring of *Populus deltoides*. In this study, the effects of genetic variation on the chemical composition, density, and anatomical characteristics were determined. The radial variation of wood property indexes was also studied to determine the rotation period of mature wood.

EXPERIMENTAL

Study Site

The experimental site was located in a plantation in Jiaozuo City, Henan Province, China (north latitude 35°10'-35°21', east longitude 113°4'-113°26'). The climate is characterized by distinct humid seasons with a temperate monsoon climate. The four seasons are distinct. The annual average temperature is 12.8 to 14.8 °C. The annual average precipitation is 600 to 700 mm. The frost-free period lasts 200 days, and there are 2200 to 2400 h per year of sunshine. Plantation trees were spaced with a 3 m × 3 m configuration. The plantation was a monoculture and the land is flat and near roads.

Plant Material and Data Collection

The female parent *Populus deltoides* cl. '55/65', the male parent *Populus deltoides* cl. '2KEN8', and their two hybrid offspring (*Populus deltoides* cl. 'Danhong' and *Populus deltoides* cl. 'Nanyang') were selected based on current production type, main planting variety, planting area, and growing conditions. The four specimens were all planted by the Research Institute of Forestry, Chinese Academy of Forestry, Jiaozuo, Henan province, which were harvested in 2017. The trees were 9 to 10 years of age, which better met the above conditions.

Trees with good growth and straight stems that were free of diseases and insect pests were selected for this study. Five plants were collected from each variety. Several discs were collected from the height of 1.3 m for each plant and air-dried. Discs were then analyzed to determine the chemical composition, main anatomical characteristics, and density of each tree. The average height of trees was between 16.7 and 19.8 m, whereas the average diameter at breast height was between 17.2 and 20.8 cm. In these samples, the average tree height and DBH (Diameter at Breast Height) of the female parent are the smallest.

Table 1. Basic Information of Parents and Offspring Progeny

Type	Age (Year)	Tree Height (m)	Diameter at Breast Height (cm)	Genetic Background	Performance growth
Female parent (F) <i>Populus deltoides</i> cl. '55/65'	10	16.8±2.9	19.6±2.3	<i>Populus deltoides</i>	Early rapid growth, excellent dry shape, Asexual reproductive ability
Male parent (M) <i>Populus deltoides</i> cl. '2KEN8'	9	16.7±1.6	17.2±1.2	<i>Populus deltoides</i>	Narrow crown, excellent dry shape, asexual reproduction ability
Offspring 1 <i>Populus deltoides</i> cl. 'Danhong'	9	19.8±1.2	20.8±0.3	Hybrid F1	Easy to survive, resistant to diseases and insects, excellent dry shape, water and humidity resistance
Offspring 2 <i>Populus deltoides</i> cl. 'Nanyang'	10	17.4±0.7	20.6±1.4	Hybrid F1	Excellent dry shape, resistant to diseases and insects

Notes: the content of tree height and DBH in the table is the average value of the 5 strains in the group is used as the test value.

Populus deltoides cl. 'Danhong' was a female plant. Under suitable site conditions, the average annual growth of DBH was as high as 6 cm, and up to 4 cm under poor site conditions. Under all kinds of site conditions, it was higher than that of the parents and the control varieties. When the susceptible rate of the varieties mixed with *Populus deltoides* cl. 'Danhong' reached more than 90%, the susceptible rate of tolerant mulberry longicorn beetles was less than 20%. Therefore, *Populus deltoides* cl. 'Danhong' was basically free from mulberry longicorn beetles or susceptible, but it could not complete the life cycle and emerged as adults. *Populus deltoides* cl. 'Nanyang' is a male plant. The average DBH for a 6-year-old was 20.70 cm, and the average tree height was 15.0 m. The susceptible rate of

the varieties mixed with *Populus deltoides* cl. 'Lux' was more than 45%, and the mulberry longicorn beetles on southern poplar could not complete their life cycle (Zhang *et al.* 2008; Hu *et al.* 2013). Basic species information is provided in Table 1.

Preparation of chemical composition samples

Lumber discs approximately 10 cm thick were cut into match-stick sizes according to the rings. The 9-year-old poplars were divided into 1 to 4 years, 5 years, 6 years, 7 years, and 8 to 9 years, and the 10-year-old poplars were divided into 1 to 4 years, 5 years, 6 years, 7 years, 8 years and 9 to 10 years (Fig. 1). A total of 40- to 60-mesh samples were taken, and the raw materials were crushed and moisture balancing took place. Three samples were taken from each group for chemical composition determination, and the results were averaged.

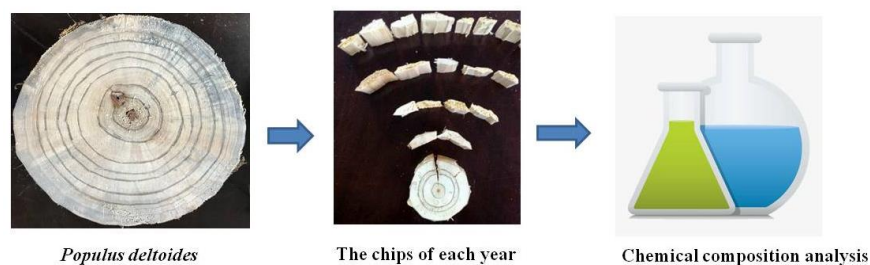


Fig. 1. The preparation process of chemical composition analysis

Density sample preparation

Lumber discs approximately 10 cm thick were collected. The end face of the polished disc was drawn and marked on the heartwood, the heartwood transition zone, and the sapwood. Then, the samples from three different regions (the heartwood, the heartwood transition zone, and the sapwood) were refined into 20 mm × 20 mm × 20 mm standard samples, with an accuracy of 0.01 mm.

Preparation of anatomical characteristic specimens

The samples for anatomical properties analyses were taken from the north side of each trunk so that the growth conditions of the studied wood were as similar as possible. Age was rechecked by counting the rings from the anatomical crosscuts at breast height; the length of strips of north side and annual ring width (mm) in the four directions (east, west, south, and north) was measured for each sample tree. Sapwood and heartwood were separated on the basis of color (Nicholls and Pederick 1979).

Measurements of Chemical Composition

The following contents were measured from the ground meal, in accordance with relative standards. The wood moisture content was determined by the gravimetric method according to the ISO 13061-1 (2014) standard. The holocellulose and α -cellulose contents were determined using sodium chlorite, according to the standard PN-P-50092 (1992). The lignin content was determined according to the TAPPI T222 om-02 (2011) standard, and benzene alcohol extractives were determined according to TAPPI T204 cm-97 (2007). The hemicellulose contents were measured as the difference between the holocellulose content and the α -cellulose content.

Measurements of Densities

The density value was measured according to the ISO 13061-2 (2014) standard.

Measurements of Anatomical Characteristics

For anatomical characterization, wood macerations were conducted using glacial acetic acid and 30% hydrogen peroxide (1:1 ratio) at 80 °C for 4 h. The macerated tissue was washed in running water until the tissue was free of all acid traces; the lengths of the fibers and vessel elements were then determined. The fibers and vessel elements were observed using a depth of field microscope (Nikon ECLIPSE Ni, Nikon Corporation, Tokyo, Japan), and images were taken at 200 magnification for analysis. In total, 50 measurements were taken for fiber length. These measurements were carried out for each annual ring from the pith to the bark.

Wood cubes ($2 \times 2 \times 2 \text{ cm}^3$) were prepared annually along radial positions from the pith to the bark. The wood blocks were softened by boiling in water with a microwave oven for approximately 15 to 20 min. Transverse, radial, and tangential microscopic sections (approximately 25-30 μm thick) were prepared with a rotary microtome Leica RM 2265 (Wetzlar, Germany). The sections were stained with safranin (5%) for approximately one minute, dehydrated with an ethanol series (50%, 70%, 80%, 90%, and 100%) for a few seconds, washed with 50% xylene and pure xylene, and mounted with a drop of Canada balsam. After drying, sections were observed using a light microscope (Nikon ECLIPSE Ni, Nikon Corporation, Tokyo, Japan) with a DS-Ri2 camera and Image NIS 5.10 software, and images were taken at 2 and 4 magnifications for analysis. ImageJ (National Institutes of Health, Bethesda, MD, USA) was used to analyze all images. 50 measurements were taken for fiber width. These measurements were carried out for each annual ring from the pith to the bark.

Wood slices with 1 mm (R) \times 10 mm (T) \times 30 mm (L) were prepared and equilibrated for 1 week in a constant temperature and humidity chamber where the temperature was 20 °C, and the relative humidity was 65%. Microfibril angle was measured by X-ray diffraction XRD-3 (XD-3, Beijing Persee Instrument Co., Ltd., Beijing, China) X-ray diffractometer (the experimental conditions: Cu target; α -ray; $\lambda=0.154 \text{ nm}$; 36 kV; 20 mA; $h=15 \text{ min}$). The data were then calculated using the 0.6 T method (Cave 1966) with Origin 2019b software. The Nikon microscope was used to measure the fiber width, which was measured randomly by selecting 50 cells for each ring in the tangential direction. The average fibre width was calculated from 20 vessels per slide.

Statistical Analysis

The means from the anatomical property data were calculated from the individual samples. Analysis of variance (ANOVA) was performed using SPSS 20.0 software (IBM Corp., Armonk, NY, USA) to determine whether there were any significant differences between clones. The ANOVA was followed by Tukey's tests at the 5% significance level to compare means. Variation in traits with cambial age was fitted with a multiple linear regression equation using OriginPro 2019b (64-bit, 9.6.5.169) software (OriginLab Corporation, v.9.6.5.169, Northampton, MA, USA).

The coefficient of variation is another statistic used to measure the degree of variation among observed values in the data. It was calculated using Eq.1,

$$\text{CV (\%)} = \frac{\sigma}{\bar{X}} \times 100 \quad (1)$$

where CV is the coefficient of variation, σ is the standard deviation of the character, and X is the mean value of the trait.

The analysis of variance uses a linear model. The purpose of variance analysis is to find out the factors that have significant influence on the thing, the interaction between the factors, and the optimal level of significant influence factors through data analysis. The actual observed value is calculated as follows,

$$Y_{ijk} = \mu + a_i + b_j + e_{ijk} \quad (2)$$

where Y_{ijk} is the actual observed value, μ is the average of the population, a_i is a clonal effect, b_j is the zone group effect, and e_{ijk} is machine error.

Genetic parameter estimation formula is the correlation between multiple measures of a trait (degree of repeatability). An index to measure the stability of their characteristics is given by,

$$R_{\text{clone}} = \frac{n\sigma_g^2}{n\sigma_g^2 + \sigma_e^2} \times 100\% \quad (3)$$

where R_{clone} is an index to measure the stability of their characteristics, σ_e^2 is the variance between different strains within the clone, environmental variance, σ_g^2 is the phenotypic variance between clones, genotype variance, and n is the number of different plants in the clone.

RESULTS AND DISCUSSION

Analysis of Chemical Composition and its Radial Variations of Parents and Offspring

Radial variation analysis of chemical composition of parents and offspring

The chemical compositions of the parents and offspring are shown in Table 2. The benzene alcohol extract content of the four samples ranged from 1.31% to 1.47% (Table 2). The extract content of *Populus deltoides* cl. ‘Danhong’ was the lowest (1.31%), and the male parent had the highest (1.47%). The extract content of *Populus deltoides* cl. ‘Nanyang’ was between the parent values, while the extract content of *Populus deltoides* cl. ‘Danhong’ showed the phenomenon of transgressive segregation. This phenomenon is characterized by quantitative phenotypes across biparental recombinant inbred, backcross or even early generation segregating populations that are beyond the parental range, *i.e.*, better than the superior parent or worse than the inferior parent (de los Reyes 2019). The difference between the offspring was only 0.09%. The holocellulose contents ranged from 82.01% to 83.57% (Table 2). The holocellulose content of the female parent was the highest (83.57%), and *Populus deltoides* cl. ‘Danhong’ was the lowest (82.01%). The holocellulose content of *Populus deltoides* cl. ‘Nanyang’ was lower than the female parent, and higher than the male parent; however, the holocellulose content of *Populus deltoides* cl. ‘Danhong’ showed the transgressive segregation phenomenon, and the difference between the offspring was 0.49%. The content of α -cellulose ranged from 46.09% to 47.25% (Table 2). The content of the female parent was the highest (47.25%), while the lowest content was from the male parent (46.05%). The α -cellulose contents from the two offspring were lower than the female parent, but higher than the male parent. The difference in the α -cellulose content between the offspring was 0.76%. The hemicellulose contents

ranged from 35.16% to 36.46% (Table 2). The hemicellulose content from *Populus deltoides* cl. 'Danhong' was the lowest (35.16%), and the female parent was the highest (36.46%). The hemicellulose content from *Populus deltoides* cl. 'Nanyang' was between the parents' values, while *Populus deltoides* cl. 'Danhong' showed the transgressive segregation phenomenon. The difference between the offspring's hemicellulose content was 0.76%. The lignin content ranged from 22.05% to 24.47% (Table 2). The lignin content was highest in the male parent (24.47%), but the female parent was the lowest (22.05%). The lignin contents of the two offspring were between the parents' values, and the lignin content difference between the offspring was 0.94%.

Table 2. Chemical Compositions of Parent and the Offsprings (%)

Type	Clones	Benzene Alcohol Extractive	Lignin	Holocellulose	α -Cellulose	Hemi-celluloses
Female Parent (F)	<i>Populus deltoides</i> cl. '55/65'	1.36 \pm 0.23	22.05 \pm 2.16	83.57 \pm 1.19	47.25 \pm 2.55	36.46 \pm 2.70
Male Parent (M)	<i>Populus deltoides</i> cl. '2KEN8'	1.47 \pm 0.14	24.47 \pm 1.41	82.28 \pm 1.13	46.09 \pm 2.18	35.79 \pm 2.16
Offspring 1	<i>Populus deltoides</i> cl. 'Danhong'	1.31 \pm 0.24	22.98 \pm 1.63	82.01 \pm 1.88	46.15 \pm 2.12	35.16 \pm 1.82
Offspring 2	<i>Populus deltoides</i> cl. 'Nanyang'	1.40 \pm 0.20	23.92 \pm 1.61	82.50 \pm 1.91	46.91 \pm 1.70	35.92 \pm 1.90

Note: The chemical composition contents in the table are benchmarked against absolutely dry wood, and the average values of the 5 strains in the group are used as test values.

The radial variation from the main chemical composition is shown in Fig. 2. The radial variation trends of the parents' benzene alcohol extract content were consistent with that of the offspring, which showed a slightly increased trend at first, but then decreased from the pith to the outside (Fig. 2a). Nault (1988) reported the same results for the content of benzene alcohol extract of *Thuja plicata* Donn. Lukmandaru and Takahashi (2009) reported that the benzene alcohol extract content of *Tectona grandis* (L.f.) decreased gradually from the pith to the outside. The radial variation trend of holocellulose content tended to be smooth from the pith to the outside, while the radial variation trend of α -cellulose content increased gradually from the pith to the outside (Fig. 2b and 2c). These results were consistent with those reported by Donata *et al.* (2019) and Zhou *et al.* (2010). The radial variation trends of the hemicellulose and lignin contents decreased gradually from the pith to the outside, and the rate of decline was slowed down for the 7- to 8-year-old plants (Fig. 2d, 2e). Xing *et al.* (1994) reported that the lignin content decreased gradually from the pith to the outside.

The linear coefficients of determination (R^2) of the cambial age with the α -cellulose, hemicellulose, and lignin of parents and offspring were 0.99, 0.99, and 0.90, respectively (Fig. 2). The p-values were all less than 0.0001. R^2 and p-values showed that the content of α -cellulose, hemicellulose, and lignin had a certain variation in the radial direction, which provided a theoretical basis for the actual production demand.

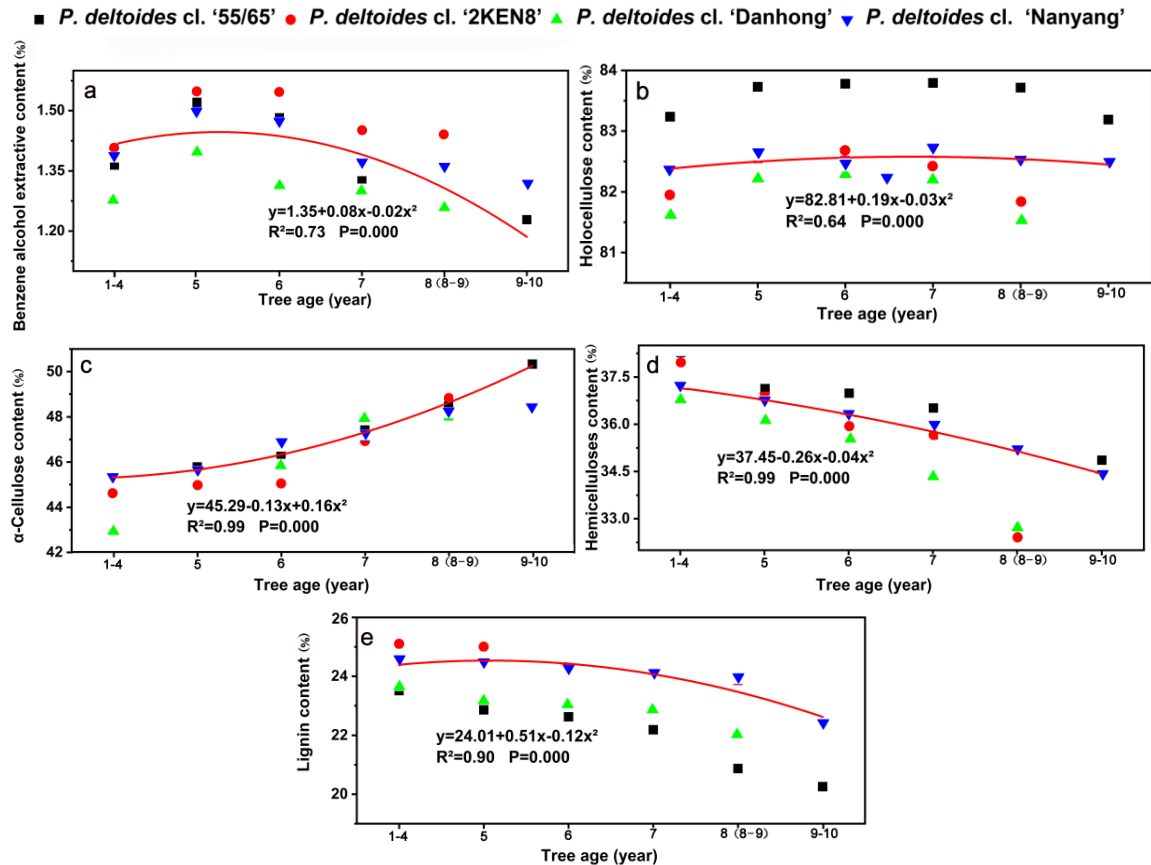


Fig. 2. Radial variation of main chemical composition: a) benzene alcohol extractive; b) holocellulose; c) α -cellulose; d) hemicellulose; e) lignin

Variance analysis of chemical components of parents and offspring

Repeatability refers to the degree of phenotypic stability of species with the same genotype at different times or places (Zhang *et al.* 2003). The repeatability of the clones is an index to measure the stability of their characteristics (Yu *et al.* 2001). Chemical composition variance analysis of the parents and offspring are shown in Table 3. The ratio of 0.6 to 0.7 showed strong inheritance, and 0.7 to 1 showed intense inheritance (Geyer *et al.* 2000). The results of the variance analysis showed that there was no significant difference in the hemicellulose contents among the four clones ($p > 0.05$), and that the growth ring had a significant effect on chemical composition ($p < 0.05$) (Table 3). It can be seen (Table 3) that the clone F-values of the benzene alcohol extract, lignin, and holocellulose were greater than that of growth rings. Therefore, the benzene alcohol extract, lignin, and holocellulose contents were greatly affected by the clones. The repeatabilities of the chemical compositions were between 0.65 and 0.85, which was controlled by strong inheritance (Table 3). The growth ring F-value of α -cellulose was greater than that of clone, indicating that the growth ring (the effect of cambial age on chemical composition characters) had a great influence on the content of α -cellulose. The repeatability of lignin, α -cellulose, and hemicelluloses was more than 0.80, suggesting that traits were strongly controlled by parental genetic control.

Table 3. Variance Analysis of Chemical Composition

Chemical Composition	Impact Factor	Sum of Squares	Degrees of Freedom	Mean Variance	F-value	Significance	Repeatability
Benzene-alcohol-extractive	clonal difference	1.074	3	0.358	8.414	**	0.68
		0.540	5	0.108	2.429	*	
Lignin	clonal difference	285.434	3	95.145	19.552	**	0.83
		92.434	5	18.487	3.366	*	
α -Cellulose	clonal difference	81.346	3	27.115	5.802	**	0.84
		692.245	5	138.449	49.146	**	
Holocellulose	clonal difference	182.643	3	60.881	24.642	**	0.79
		146.705	5	29.341	11.299	**	
Hemicellulose	clonal difference	31.887	3	10.629	2.219	**	0.87
		591.126	5	118.225	38.209	**	

** Indicates significant difference at $\alpha = 0.01$ level; * indicates significant difference at $\alpha = 0.05$ level;

Density Analysis and Radial Variation of Parents and Offspring

Radial variation analysis of parent and offspring density

Parent and offspring densities are shown in Table 4. Radial variation of the densities is shown in Fig. 3. The basic parents and offspring densities were 0.37 to 0.43 g/cm³. The air-dry densities were 0.44 to 0.51 g/cm³. The absolute dry densities were 0.42 to 0.49 g/cm³ (Table 4). The basic densities, air-dry densities, and absolute dry densities of *Populus deltoides* cl. ‘Nanyang’ were the highest, while those of the male parents were the lowest. According to the variation law of densities between parents and offspring, the density value of *Populus deltoides* cl. ‘Danhong’ was between the parent values, while *Populus deltoides* cl. ‘Nanyang’ showed the transgressive segregation phenomenon. The density variations gradually increased with increased cambial age from pith to the outside (Fig. 3). These results are similar to the results of Pliura *et al.* (2007) and Lachenbruch *et al.* (2011); however, Bao *et al.* (2001) reported that the density of cork oak trees decreased gradually from pith to bark.

The linear coefficients of determination (R^2) of the cambial age with the basic density, air-dry density and absolute dry density of the parents and offspring were all higher than 0.84, and the p-values were all less than 0.0001. The density increased in varying degrees at each stage in the radial direction (Fig. 3).

Table 4. Densities of Parent and Hybrids (g·cm⁻³)

Type	Clones	Basic Density	Air Dry Density	Absolute Dry Density
Female Parent (F)	<i>Populus deltoides</i> cl. '55/65'	0.39 ± 0.04	0.48 ± 0.05	0.45 ± 0.04
Male Parent (M)	<i>Populus deltoides</i> cl. '2KEN8'	0.37 ± 0.03	0.44 ± 0.03	0.42 ± 0.03
Offspring 1	<i>Populus deltoides</i> cl. 'Danhong'	0.38 ± 0.03	0.45 ± 0.03	0.43 ± 0.04
Offspring 2	<i>Populus deltoides</i> cl. 'Nanyang'	0.43 ± 0.03	0.51 ± 0.04	0.49 ± 0.04

Note: The densities' average values of the 5 strains in the group are used as test values.

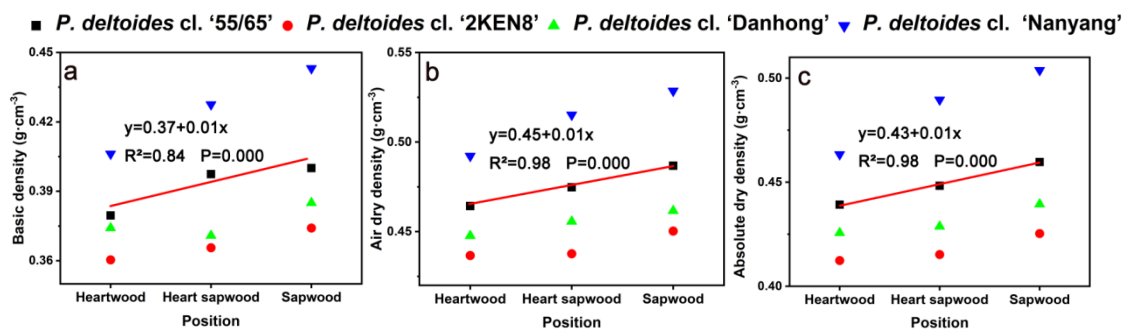


Fig. 3. Radial variation of density: a) basic density; b) air dry density; c) absolute dry density

Variance analysis of parent and offspring density

Density variance analysis is shown in Table 5. The results of the variance analysis showed that the differences between the clones and the position from the pith had a highly significant effect on wood density ($p < 0.01$). From the F-values, it can be seen that the basic, air-dry, and absolute dry densities from clones were greater than that of position difference, indicating that clones had a great influence. When selecting materials, clones were the key properties of wood density considered (Table 5).

Table 5. Variance Analysis of Density

Parameters	Impact Factor	Sum of Squares	Degrees of Freedom	Mean Variance	F-value	Significance
Basic Density	Clones	0.574	3	0.19	185.65	**
	Position	0.061	2	0.03	20.36	**
Air Dry Density	Clones	0.814	3	0.271	188.14	**
	Position	0.045	2	0.022	10.35	**
Absolute Dry Density	Clones	0.743	3	0.248	175.42	**
	Position	0.061	2	0.03	14.828	**

** Indicates significant difference at $\alpha = 0.01$ level; * indicates significant difference at $\alpha = 0.05$ level;

Anatomical Characteristics Analysis and Radial Variation of Parents and Offspring

Radial variation of microfibril angle, fibre length, and fibre width

Radial variation of the main anatomical characteristics are shown in Fig. 4, and the values are presented in Table 6. The microfibril angle of *Populus deltoides* cl. ‘Nanyang’ was the largest (17.62°), which displayed the transgressive segregation phenomenon, and the smallest was the female parent, *Populus deltoides* cl. ‘55/65’ (16.52°). The fibre lengths of the parents and offspring were 1026 to 1153 μm (Table 6). The longest fibre length was *Populus deltoides* cl. ‘Nanyang’ (1153 μm), which showed the transgressive segregation phenomenon. The shortest fibre length was the female parent (1026 μm). The fibre widths of the parents and offspring ranged from 13.79 to 17.78 μm (Table 6). The maximum fibre width was 17.78 μm for *Populus deltoides* cl. ‘Danhong’, and the minimum was 13.79 μm for the male parent. The microfibril angles of the parents and offspring decreased gradually with the increase of cambial age, and the decreasing speed slowed down at 7 to 8 years (Fig. 4a). The fibre length increased gradually with increased cambial age and the increasing speed slowed down slightly at 7 to 8 years (Fig. 4b). The fibre width increased gradually with the increase of cambial age, while it decreased after 7 years (Fig. 4c). Koubaa *et al.* (1998) reported that the fibre lengths increased gradually.

The changes in fibre length and width were related to the maturity of the tree cells. The trees near the pith were young with immature cells, and the cells divided fast and grew fast. When trees reached the maturity stage, the cells were relatively stable and divided slowly. The changes in cell length were also relatively stable and the growth rate of the trees decreased. This is the reason that the increase in fibre length and width slowed down in 7- to 8-year-old plants (Pitre *et al.* 2007).

The linear coefficients of determination (R^2) of the cambial age with the microfibril angle and the fibre length of the parents and offspring were 0.97 and 0.94, respectively, and the p-values were less than 0.0001 (Fig. 4). The changes in microfibril angle and fibre length in the radial direction were the internal reasons for the properties of wood microdissection materials.

Radial variation of ratio of fibre length to width, wall thickness to lumen ratio, and ratio of lumen diameter to wall thickness

It can be seen that the ratio of fibre length to width in the parents and offspring were 63.99 to 74.46 (Table 6). The ratio of fibre length to width of *Populus deltoides* cl. ‘Danhong’ and *Populus deltoides* cl. ‘Nanyang’ were lower than their parents due to the transgressive segregation phenomenon. The largest ratio of fibre length to width was in the male parent (74.46), while the lowest ratio was in *Populus deltoides* cl. ‘Danhong’ (63.99). The wall thickness to lumen ratios were 0.26 to 0.39, and both offspring had the transgressive segregation phenomenon (Table 6). The wall thickness to lumen ratio of *Populus deltoides* cl. ‘Nanyang’ was the largest (0.39), and *Populus deltoides* cl. ‘Danhong’ was the smallest (0.26). The ratio of lumen diameter to wall thickness was 0.73 to 0.80, and both offspring had the transgressive segregation phenomenon (Table 6). The ratio of lumen diameter to wall thickness of *Populus deltoides* cl. ‘Nanyang’ was the smallest (0.73), while *Populus deltoides* cl. ‘Danhong’ was the largest (0.80).

The linear coefficients of determination (R^2) of the tree age with the microfibril angle, fibre length were all higher than 0.84, while the fibre width and ratio of fiber length to width were higher than 0.70. The R^2 value of wall thickness to lumen ratio and ratio of lumen diameter to wall thickness of the parents and offspring were only 0.27, and the p-

values were all less than 0.0001. The ratio of fibre length to width increased gradually with the increase of tree age, and then decreased at the 8th year (Fig. 4d). The wall/lumen ratio decreased at first, and then increased with the increase of tree age, and exhibited the lowest value at 7 years (Fig. 4e). The ratio of fiber lumen diameter to wall thickness increased at first, then decreased with the increase of tree age, and reached its highest at 7 years as shown in Fig. 4f.

■ *P. deltoides* cl. '55/65' ● *P. deltoides* cl. '2KEN8' ▲ *P. deltoides* cl. 'Danhong' ▼ *P. deltoides* cl. 'Nanyang'

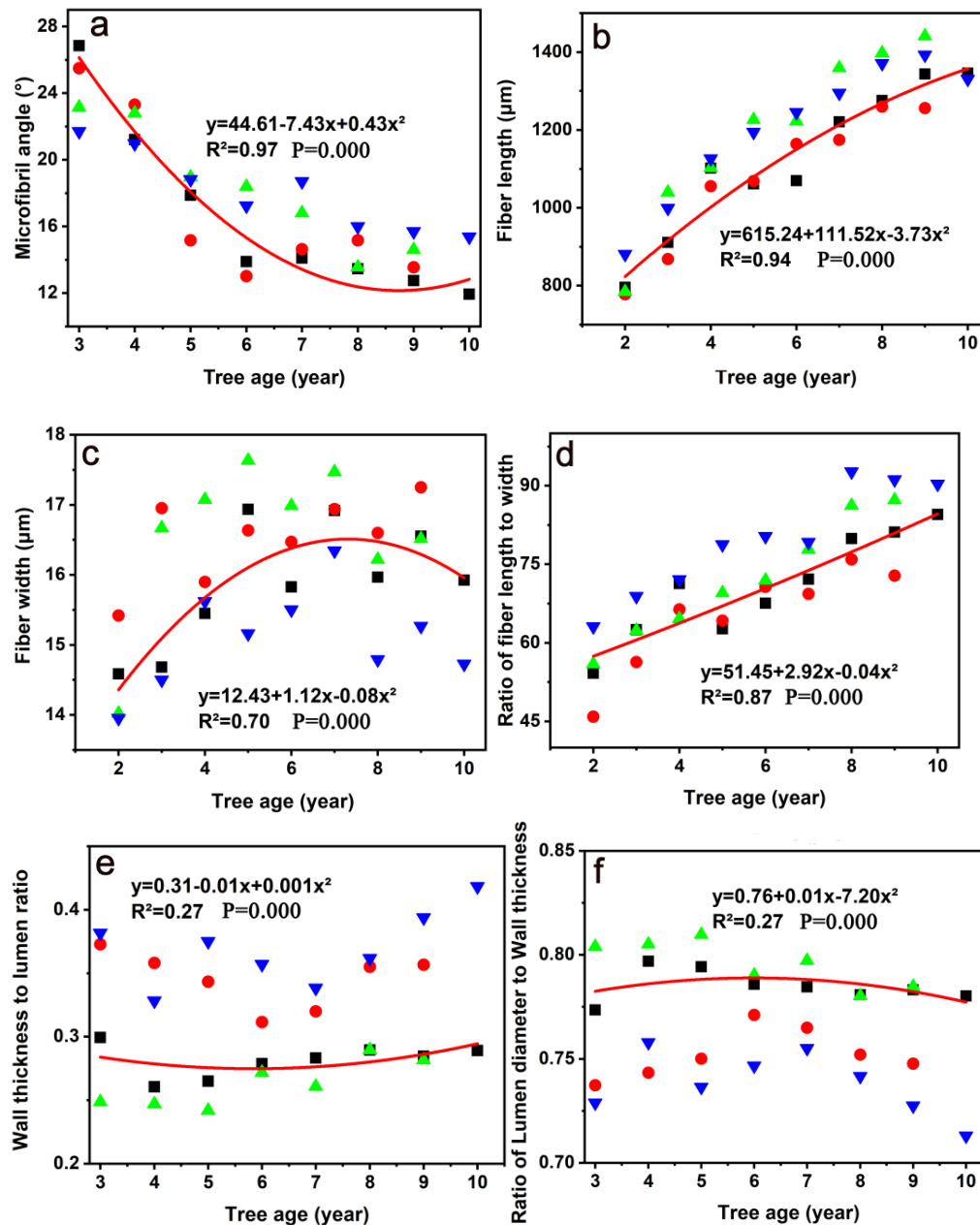


Fig. 4. Radial variation of main anatomical characteristics: a) microfibril angle; b) fiber length; c) fiber width; d) ratio of fiber length to width; e) wall thickness to lumen ratio; f) ratio of lumen diameter to wall thickness

Table 6. Main Anatomical Characteristics of Parents and Hybrid

Type	Clones	Microfibril Angle (°)	Fibre length (µm)	Fibre Width (µm)	Ratio of Fiber Length to Width	Wall Thickness to Lumen Ratio	Ratio of Lumen Diameter to Wall Thickness
Female Parent (F)	<i>Populus deltoides</i> cl. '55/65'	16.52 ± 5.27	1086.16 ± 274.03	15.95 ± 2.99	68.09 ± 3.25	0.29 ± 0.12	0.79 ± 0.07
Male Parent (M)	<i>Populus deltoides</i> cl. '2KEN8'	17.27 ± 6.72	1026.75 ± 266.16	13.79 ± 1.60	74.46 ± 2.67	0.36 ± 0.11	0.75 ± 0.06
Offspring 1	<i>Populus deltoides</i> cl. 'Danhong'	17.10 ± 5.68	1137.76 ± 298.94	17.78 ± 2.14	63.99 ± 3.26	0.26 ± 0.09	0.80 ± 0.05
Offspring 2	<i>Populus deltoides</i> cl. 'Nanyang'	17.42 ± 6.21	1152.90 ± 262.19	17.30 ± 1.51	66.64 ± 3.21	0.39 ± 0.13	0.73 ± 0.07

Note: The main anatomical characteristics in the table are benchmarked against air-dried wood, and the average values of the 5 strains in the group are used as test values.

The ratio of fibre length to width, the wall thickness to lumen ratio, and the ratio of fiber lumen diameter to wall thickness were related to the differentiation and growth of cells. During the process of differentiation and fibre cell growth, the cell cavity was enlarged, the cell wall thickened, and cell growth was mainly pseudo-lateral growth, which led to cell lumen enlargement and cell limited wall thickening. Because the cambium primordial cells underwent mainly pseudo-lateral growth in the process of differentiation, the fibre length increased rapidly in the transition from infancy to maturity. The fibre width (including cell cavity and cell wall thickness) increased slowly and less.

Variance of main anatomical characteristics analysis of parents and offspring

Variance analyses of the anatomical characteristics are shown in Table 7. The results indicate that the clones had a significant effect on fibre length, fibre width, ratio of fibre length to width, wall thickness to lumen ratio, and ratio of lumen diameter to wall thickness ($p < 0.01$), and had no significant effect on microfibril angle (Table 7). The effects of growth rings on the anatomical characteristics were significant ($p < 0.05$). The F-values indicated that the values of fibre width, wall thickness to lumen ratio, and ratio of lumen diameter to wall thickness for clones were greater than that for growth ring, suggesting that the fibre width, wall thickness to lumen ratio, and ratio of lumen diameter to wall thickness were greatly influenced by clones. The values of fibre length and ratio of fibre length to width for growth ring were greater than clone. The fibre length and ratio of fibre length to width were greatly influenced by growth ring.

Table 7. Variance Analysis of Main Anatomical Characteristics

Parameters	Impact Factor	Sum of Squares	Degrees of Freedom	Mean Variance	F-value	Significance
Microfibril Angle	Clones Growth ring	14.385 1471.238	3 8	4.80 183.91	0.20 11.96	>0.05 **
Fiber Length	Clones Growth ring	22722026.03 464117205.40	3 8	7574008.67 51568578.38	99.93 1757.85	** **
Fiber Width	Clones Growth ring	18304.115 10049.759	3 8	3050.68 1256.22	238.18 95.14	** **
Ratio of Fiber Length to Width	Clones Growth ring	1217.487 3537.294	3 8	405.83 442.16	3.29 7.99	* **
Wall Thickness to Lumen Ratio	Clones Growth ring	13.499 1.621	3 7	4.48 0.23	564.44 24.09	** **
Ratio of Lumen Diameter to Wall Thickness	Clones Growth ring	3.807 0.465	3 7	1.27 0.66	599.27 25.65	** **

** Indicates significant difference at $\alpha = 0.01$ level; * indicates significant difference at $\alpha = 0.05$ level;

Analysis on the Overall Variation of Wood Properties between Parents and Offspring

Analyses of the overall variation in the wood properties are shown in Table 8.

Table 8. Analysis of the Overall Variation of Wood Properties

Parameters	Mean	Standard Deviation	Coefficient of Variation (%)	Confidence Interval at 95%	
				Lower	Upper
Benzene-alcohol extractive	1.39	0.21	15.11	1.36	1.41
Holocellulose	82.59	1.73	2.09	82.18	82.56
α -Cellulose	46.64	2.21	4.74	46.40	46.88
Hemicelluloses	35.83	2.20	6.14	35.49	35.96
Lignin	23.32	2.39	10.25	23.06	23.58
Basic Density	0.39	0.04	10.26	0.38	0.39
Air Dry Density	0.47	0.05	10.64	0.46	0.47
Absolute Dry Density	0.45	0.05	11.11	0.44	0.45
Microfibril Angle	16.83	4.46	26.50	16.13	17.53
Fiber Length	1101.87	279.58	25.37	1096.25	1107.49
Fiber Width	16.50	3.43	20.79	16.46	16.55
Ratio of Fiber Length to Width	67.79	12.12	17.88	63.49	72.09
Wall Thickness to Lumen Ratio	0.31	0.10	32.26	0.31	0.32
Ratio of Lumen Diameter to Wall Thickness	0.77	0.05	6.49	0.76	0.77

The wood properties with coefficients of variation less than 10% were holocellulose, α -cellulose, hemicelluloses, and the ratio of lumen diameter to wall thickness within the respective 95% confidence interval, which were relatively stable and conducive to genetic control (Table 8). In the variation analysis of the material properties, the coefficient of variation of wall thickness to lumen ratio was the largest (32.26%), while the coefficient of variation of holocellulose was the smallest (2.09%) (Table 8).

CONCLUSIONS

1. The chemical contents (benzene alcohol extract, holocellulose, and hemicellulose) in offspring (*Populus deltoides* cl. ‘Danhong’) showed the transgressive segregation phenomenon. The density of *Populus deltoides* cl. ‘Nanyang’ showed the transgressive segregation phenomenon. Furthermore, the anatomical characteristics of the two offspring showed transgressive segregation phenomenon (except that the microfibril angle of *Populus deltoides* cl. ‘Danhong’ was between the parent values). The clones had no significant effect on the differences in hemicellulose and microfibril angle but had a significant effect on the other wood properties. The growth rings (the position from the pith) had a significant effect on the wood properties. Through the variance analyses, it can be concluded that the differences of each character between parents and offspring were different.
2. The law of radial variation showed that the radial variation of the parents was consistent with that of the offspring. At the age of 7 to 8 years, the decreasing rate of benzene alcohol extract, hemicellulose, lignin, microfibril angle, and ratio of lumen diameter to wall thickness decreased slowly, while α -cellulose, fibre length, fibre width, ratio of fibre length to width, and wall thickness to lumen ratio increased slowly, and the density increased gradually from pith to bark.
3. In summary, the offspring of *Populus deltoides* cl. ‘Danhong’ showed the transgressive segregation phenomenon in chemical composition and anatomical characteristics, while *Populus deltoides* cl. ‘Nanyang’ showed the transgressive segregation phenomenon in density and anatomical characteristics. To select high quality germplasm resources through genetic breeding, the variability of the clones and their wood properties must be considered in the process of breeding. According to the rule of radial variation of wood properties, the rotation period can be selected in the range 7 to 8 years.

ACKNOWLEDGMENTS

This research was funded by the National Key Research and Development Program of China (No. 2017YFD0600201) and the International Science and Technology Cooperation Program of Anhui Province (1704e1002226).

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Article submitted: January 26, 2021; Peer review completed: March 21, 2021; Revised version received and accepted: April 29, 2021; Published: May 17, 2021.

DOI: 10.15376/biores.16.3.4905-4923