Agronomic performance of 27 *Populus* clones evaluated after two 3-year coppice rotations in Henan, China

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Abstract

Selecting superior clones is the first step for commercial short-rotation coppice cultures to provide biomass and bioenergy. Till date, such selection for hybrid *Populus* clones in middle China is absent. Here we describe the growth, aboveground biomass production and cell wall composition of 27 hybrid poplar clones in Henan, China for two 3-year rotations. Significant variation in these three characteristics over two triennial rotation coppices among the 27 poplar clones was observed. During two 3-year rotation coppices, clones ‘276’, ‘02-17’, and ‘599’ showed relatively higher tree heights and larger basal diameters than those of the other clones. However, the most productive clones were ‘36’ and ‘01-30’. At the end of the second triennial rotation, the aboveground biomass production reached 18 Mg ha⁻¹ year⁻¹. For the cell wall composition analysis, the cellulose contents of clones ‘01-243’ and ‘2001’ were relatively high, while the xylose contents of clones ‘01-30’ and ‘65’ were relatively high. Cluster analysis based on height, basal diameter, biomass, heat value, cellulose content, and survival rate revealed five growth potential classes. Accordingly, clones ‘03-332’, ‘36’, and ‘599’ exhibited high biomass and growth and had the greatest potential to serve as excellent biomass producers in Henan, China. In addition, the expression patterns of 20 key regulatory genes were analyzed, and an integrated coexpression network was constructed. This field trial provides a comprehensive quantification and evaluation of the agronomic performance of 27 poplar clones in Henan, China. The results of this study and the analytical strategies provide an efficient mechanism for selecting clones that will perform well agronomically in local environments. The expression of key genes and the integrated coexpression network provide the molecular mechanisms of poplar biomass performance.

Keywords

biofuels, biomass, cell wall, field trial, growth, integrated regulatory network, *Populus*, short-rotation coppice, yield
INTRODUCTION

China is a developing country with limited forest resources that cannot meet the increasing demand of timber and fuel. Short-rotation coppice (SRC) culture could provide wood chips and has been considered a flexible way to fill the gap between biomass and bioenergy demand and supply (Parikk, 2004). Therefore, the SRC culture in China developed rapidly (Guo & Zhang, 2010). Willows (Salix spp.), eucalypts (Eucalyptus spp.), black locusts (Robinia pseudoacacia L.) and poplars (Populus spp.) are commonly used SRC trees (Paris et al., 2011), owing to their rapid, early growth rates as well as their relatively large woody biomass production. Among them, poplar species are the most suitable SRC trees in China, considering their wide distribution and the substantial genetic variation (Deckmyn, Laureysens, Garcia, Muys, & Ceulemans, 2004).

For SRC, the first and most important thing is to evaluate clonal performance and select superior clones (Verlinden, Broeckx, Bulcke, Acker, & Ceulemans, 2013). The common selection criteria include high growth vigor, high biomass production and disease resistance (Ceulemans & Deraedt, 1999). Recently, more attention has been paid to the chemical composition of biomass since the qualitative traits of harvestable biomass and its conversion into second generation biofuels (e.g., bioethanol) have become more attractive (Guidi, Tozzini, & Bonari, 2009). SRC culture of Populus has been carried out in China for several years (Gong, Zhang, & Huang, 2011; Guo & Zhang, 2010). Early in 2010, Huang’s group evaluated the performance of 14 hybrid poplar clones imported from United States after three growing seasons in northern China and the clones suitable as biomass producers were selected based on their survival and tree volume index (Guo & Zhang, 2010). Later, in 2011, the same group investigated the ecophysiological and morphological characteristics of 14 North American poplar clones in short-rotation plantations through two growing seasons and evaluated their potential suitability for use in northern China (Gong et al., 2011). In summary, the establishment of Populus suitable for SRC was executed in northern China, and the poplar clones used were imported clones. In China, several introduced poplar species and interspecific hybrids, such as Populus deltoides ‘Danhong’, P. deltoides ‘Nanyang’, P. deltoides ‘Beiyang’, P. deltoides ‘Nankang’, P. deltoides ‘Chuangxin’, P. maximowiczii ‘Eridano’, P. deltoides ‘55/56’, P. deltoides ‘2KEN8’, and P. × euramericana ‘Sangju’ demonstrated fast-growth characteristics and increased adaptability (Cao, Li, Li, & Hu, 2016; Hu et al., 2013; Wang et al., 2017; Zhang, Qixiang, Jinxing, Qihua, & Lixun, 2009; Zhao, Zhang, Li, Han, & Hu, 2008). Considering the economic benefits, it is worth developing SRC using fast-growth poplar clones. With rapid increase in biomass demand in China, developing SRC will promote local economic development.

MATERIALS AND METHODS

2.1 Experimental field description and plant material

The experimental field is in Jiaozuo, Henan Province, China (35°8′N, 113°16′E; Figure S1a). The field was established on flat agricultural soil 91 m above sea level with wheat as the main cultivated crop. The climate is temperate continental with an annual average temperature of 15.2°C, a lowest temperature of −14.3°C and a highest temperature of 43.6°C. The annual rainfall is 625.4 mm, with approximately 69% occurring from June to September, and the frost-free period is as long as 224 days. The soil quality is sandy texture with organic carbon and total nitrogen contents of 1.58% and 0.09%, respectively. The soil pH is approximately 7.2. The climate changes from 2006 to 2013 in the experimental field are shown in Figure S1.

A total of 27 hybrid poplar clones were planted on 16 and 17 March 2007. All 27 genotypes were obtained from the Research Institute of Forestry, Chinese Academy of Forestry (Beijing). The poplar genotypes represented different species and the interspecific hybrids of P. deltoides.
‘Danhong’, *P. deltoides* ‘Nanyang’, *P. deltoides* ‘Beiyang’, *P. deltoides* ‘Nankang’, *P. deltoides* ‘Chuangxin’, *P. maximowiczii* ‘Eridano’, *P. deltoides* ‘55/56’, *P. deltoides* ‘2KEN8’, and *P. × euramericana* ‘Sangju’. The interspecies hybrids used in this study were superior selections based on biomass and pest/disease resistance from field. Details on the origin and the parentage of the 27 genotypes are shown in Table 1.

Preceding the planting, soil preparation included plowing (30–40 cm depth), tilling and applying a pre-emergent herbicide treatment in autumn 2006. In March 2007, cuttings (15 cm long) of each clone were planted by hand. All the plantations were established with a randomized block design, and each clone was replicated in three separate plots. Each plot was planted in a planting scheme with 6 rows × 6 lines and a distance of 1 m, corresponding to a tree density of

**Table 1** Place of origin, botanical and parental characteristics of the 27 poplar (*Populus*) genotypes used in this study. D = *Populus deltoides*; M = *Populus maximowiczii*; N = *Populus nigra*; E = *P. × euramericana*

| Clone | Parentage | Section | Gender | Pedigree | Latin name | Year of cross/commercialization |
|-------|-----------|---------|--------|----------|------------|-------------------------------|
| 01-104 | D × D     | Aigeiros | Male   | *P. deltoides* ‘Danhong’ × *P. deltoides* ‘Nanyang’ | *P. deltoides* | 2003/                          |
| 01-202 | D × D     | Aigeiros | Female | *P. deltoides* ‘Danhong’ × *P. deltoides* ‘Nanyang’ | *P. deltoides* | 2003/                          |
| 01-243 | D × D     | Aigeiros | Male   | *P. deltoides* ‘Danhong’ × *P. deltoides* ‘Nanyang’ | *P. deltoides* | 2003/                          |
| 01-275 | D × D     | Aigeiros | Male   | *P. deltoides* ‘Danhong’ × *P. deltoides* ‘Nanyang’ | *P. deltoides* | 2003/                          |
| 01-30  | D × D     | Aigeiros | Male   | *P. deltoides* ‘Danhong’ × *P. deltoides* ‘Nanyang’ | *P. deltoides* | 2003/                          |
| 02-10  | D × D     | Aigeiros | Male   | *P. deltoides* ‘Danhong’ × *P. deltoides* ‘Beiyang’ | *P. deltoides* | 2003/                          |
| 02-17  | D × D     | Aigeiros | Male   | *P. deltoides* ‘Danhong’ × *P. deltoides* ‘Beiyang’ | *P. deltoides* | 2003/                          |
| 03-176 | D × D     | Aigeiros | Male   | *P. deltoides* ‘Danhong’ × *P. deltoides* ‘Beiyang’ | *P. deltoides* | 2003/                          |
| 03-332 | D × D     | Aigeiros | Male   | *P. deltoides* ‘Danhong’ × *P. deltoides* ‘Chuangxin’ | *P. deltoides* | 2003/                          |
| 04-107 | D × D     | Aigeiros | Male   | *P. deltoides* ‘Nankang1A’ × *P. deltoides* ‘Beiyang’ | *P. deltoides* | 2003/                          |
| 04-143 | D × D     | Aigeiros | Male   | *P. deltoides* ‘Nankang1A’ × *P. deltoides* ‘Beiyang’ | *P. deltoides* | 2003/                          |
| 04-95  | D × D     | Aigeiros | Male   | *P. deltoides* ‘Nankang1A’ × *P. deltoides* ‘Beiyang’ | *P. deltoides* | 2003/                          |
| 05-33  | D × D     | Aigeiros | Male   | *P. deltoides* ‘Nankang1A’ × *P. deltoides* ‘Chuangxin’ | *P. deltoides* | 2003/                          |
| 110    | D × M     | Aigeiros | Female | *P. deltoides* × *P. maximowiczii* ‘Eridano’ | *P. deltoides* × *P. maximowiczii* ‘Eridano’ | 1955/1984                     |
| 136    | D × D     | Aigeiros | Male   | *P. deltoides* ‘55/56’ × *P. deltoides* ‘2KEN8’ | *P. deltoides* | 1992/                          |
| 162    | D × D     | Aigeiros | Male   | *P. deltoides* ‘55/56’ × *P. deltoides* ‘2KEN8’ | *P. deltoides* | 1990/                          |
| 2001   | E         | Aigeiros | Female | *P. deltoides* × *P. nigra* | *P. × euramericana* ‘2001’ | 2001                           |
| 276    | D × D     | Aigeiros | Male   | *P. deltoides* ‘55/56’ × *P. deltoides* ‘2KEN8’ | *P. deltoides* | 1990/2003                      |
| 313    | D × D     | Aigeiros | Female | *P. deltoides* ‘55/56’ × *P. deltoides* ‘2KEN8’ | *P. deltoides* | 1990/                          |
| 36     | D         | Aigeiros | Male   | *P. deltoides* | *P. deltoides* ‘2KEN8’ | 1969/1980                      |
| 50     | D         | Aigeiros | Female | *P. deltoides* | *P. deltoides* ‘55/56’ | /1981                          |
| 599    | D × D     | Aigeiros | Male   | *P. deltoides* ‘Danhong’ × *P. deltoides* ‘Nanyang’ | *P. deltoides* | 2003/                          |
| 65     | D × D     | Aigeiros | Female | *P. deltoides* ‘55/56’ × *P. deltoides* ‘2KEN8’ | *P. deltoides* | 1992/                          |
| 910-95 | E         | Aigeiros | Male   | *P. × euramericana* ‘Sangju’ | *P. × euramericana* ‘Sangju’ | /1998                          |
| Be     | E         | Aigeiros | Male   | *P. × euramericana* ‘Bellini’ | *P. × euramericana* ‘Bellini’ | /1968/1980                     |
| G2     | E         | Aigeiros | Female | *P. × euramericana* ‘Jier’ | *P. × euramericana* ‘Jier’ | /1998                          |
| N179   | N         | Aigeiros | Male   | *P. nigra* | *P. nigra* | /2005                          |
approximately 10,000 ha\(^{-1}\). Trees were irrigated as needed in the arid growing season. Weeds were controlled artificially throughout the study.

As shown in Figure 1a, all trees were coppiced 5 cm above ground level with a gasoline chain saw during February 2008 to promote multiple stem production. After the first three years of growth (2008–2010), that is, at the end of rotation 1, the plantation was harvested manually for the first time in February 2011. From that time on, trees continued to grow as a coppice culture in the following triennial rotation (2011–2013), that is, rotation 2. The second harvest took place in February 2014.

2.2 | Growth measurement

To prevent edge effects, data collection focused only on the central row of each plot, with a sampling area of 4 rows × 4 lines. The number of living plants was assessed in each sampling area in June 2010 and June 2013 as survival percentage (Figure 1b). Basal diameter (measured at 5 cm above the soil level) and stem height were recorded (average value if plants have multiple stems) at the end of the first and second rotation on all trees in each sampling area.

2.3 | Biomass measurement

At the end of each rotation, 16 trees of each clone were selected and harvested at 5 cm above ground level. The moisture content of harvested trees was estimated based on the fresh weight and dry weight of the samples collected from three randomly selected trees in each plot. Samples were dried at 85°C until a constant mass was reached, and biomass production was calculated on an oven dry weight per ha basis.

2.4 | Heat value measurement

The heat value was determined for ground oven-dried samples. Subsamples of stems repackaged from the 27 poplar clones were ground into fine powder. Pellets were made in a special device, which produced pellets ranging from 0.7 to 0.8 g. Samples were combusted in a Parr 6300 adiabatic calorimeter. Correction factors for the formation of acids were not included in the gross heat of combustion (higher heating value) calculations. There were three field replications for each sample.

2.5 | Cell wall composition analyses

The monosaccharide assays were performed as described previously (Li et al., 2009). In brief, the stems of 27 poplar clones were ground into fine powder. Then, the powder was successively treated with 70% ethanol, a mixture of chloroform and methanol (1:1 v/v), pullulanase M1 (Megazyme) and \(\alpha\)-amylase (Sigma) in 0.1 M NaOAc buffer (pH 5.0) to obtain the destarched alcohol insoluble residues (AIRs). Two milligrams of destarched AIR samples was hydrolyzed in 2 M trifluoroacetic acid (TFA) at 121°C for 90 min and then, the released sugars were reduced by sodium borohydride (10 mg/ml in 1 M ammonium hydroxide) to generate the alditol acetates. Finally, the derivatives were analyzed using an Agilent 7890 series GC equipped with a 5975C MS.
detector (Agilent). The crystalline cellulose content was obtained by hydrolyzing the remains of TFA with Updegraff reagent (acetic acid:nitric acid:water, 8:1:2 v/v) at 100°C for 30 min. Then, the pellet was treated with 72% sulfuric acid, and the content was quantified using an anthrone assay. Acid-insoluble lignin (%, w/w) was measured according to the Chinese standard GB/T 2677.8-1994. Three field replicates were included in this experiment. At least 12 samples from separate trees within each replicate were used for each replication.

2.6 | RNA extraction and qRT-PCR

For gene expression analysis, the developing xylem that was scraped using razor blades from the bark-removed stem was collected and immediately frozen in liquid nitrogen and stored at −80°C for further analysis. Total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen) with on-column treatment with RNase-free DNase I (Qiagen) to remove genomic DNA. First-strand cDNA synthesis was carried out with approximately 1 μg RNA using the SuperScript III reverse transcription kit (Invitrogen) according to the manufacturer’s procedure. For qRT-PCR, the primers were designed using Primer3 (http://frodo.wi.mit.edu/primer3/input.htm) with a melting temperature of 58–60°C and a production of 150–250 bp. The primers used in this study are listed in Table S1. qRT-PCR was performed using a SYBR Premix Taq Kit (TaKaRa) and conducted on a LightCycler 480 Detection System (Roche). The expression was compared with the expression in clone ‘N179’ (set as 1). Relative expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method (Livak & Schmittgen, 2001). The PtUbq and PtTubulin genes were used as internal controls. All experiments were performed in three biological replicates.

2.7 | Statistical analysis

Data analyses were performed using IBM SPSS Statistics 20.0 (IBM). Comparisons of plot means for the different traits among clones were performed with the Duncan post hoc tests.

3 | RESULTS

The performance of the 27 hybrid poplar clones was evaluated over two coppice triennial rotations (Figure 1a). The survival rate, height, basal ground cover and biomass were measured at the end of each triennial rotation coppice, and the heat value and the cell wall component were measured at the end of the first triennial rotation coppice.

3.1 | Survival rate

At the end of the first rotation, the rooting rate of the clones was relatively steady at above 75% ($M = 88.7\%$). However, at the end of the second rotation, the survival rate of the clones varied significantly, ranging from 29.17% to 81.25% ($M = 58.6\%$, Figure 1b). The rooting rate and the survival rate were not comparable for a specific clone. For example, clone ‘313’ had a relatively high rooting rate of 89.58%, but its survival rate was very low. The clonal rank of the survival rate for most clones was relatively steady. Clone ‘36’ showed significantly lower survival rates than the other clones, while clone ‘01-243’ displayed the highest survival rate.

3.2 | Growth

Tree height and basal diameter are the two major indicators for describing plant growth vigor. We measured these two factors at the end of the two triennial rotations. Tree heights at the end of the first rotation were an average of 3.72 m and ranged from 3.12 m (clone ‘50’) to 4.65 m (clone ‘36’; Figure 2a). For the basal diameter, the average was 2.60 cm, and the range was 2.12 cm (clone ‘110’) to 3.45 cm (clone ‘36’; Figure 2b). At the end of the second triennial rotations, the tree heights both demonstrated a greater improvement than that in the first rotation, as the tree heights ranged from 5.67 m (clone ‘110’) to 7.73 m (clone ‘02-17’), with an average of 6.59 m (clone ‘36’; Figure 2a). For the basal diameter, the average was 2.60 cm, and the range was 2.12 cm (clone ‘110’) to 3.45 cm (clone ‘36’; Figure 2b). At the end of the second triennial rotations, the tree height and the basal diameter both demonstrated a greater improvement than that in the first rotation, as the tree heights ranged from 5.67 m (clone ‘110’) to 7.73 m (clone ‘02-17’), with an average of 6.59 m (Figure 2a), and the basal diameter ranged from 3.4 cm (clone ‘110’) to 6.34 cm (clone ‘02-17’), with an average of 4.57 cm (Figure 2b).

The clonal ranking of tree height and basal diameter at the end of the first rotation differed from that of the second rotation. Regarding tree height, clones ‘36’, ‘276’, ‘01-275’, ‘01-202’, and ‘04-107’ were the top five ranked clones at the end of the first rotation, while clones ‘02-17’, ‘Be’, ‘136’, ‘276’, and ‘01-30’ were the top five ranked clones at the end of the second rotation. Only clone ‘276’ demonstrated stable, rapid longitudinal growth over the two triennial rotations. Regarding the basal diameter, clones ‘36’, ‘599’, ‘01-104’, ‘02-17’, and ‘01-202’ were the top five ranked clones at the end of the first rotation, while clones ‘02-17’, ‘Be’, ‘136’, ‘276’, and ‘01-30’ were the top five ranked clones at the end of the second rotation. Only clone ‘276’ demonstrated stable, rapid longitudinal growth over the two triennial rotations. Regarding the basal diameter, clones ‘36’, ‘599’, ‘01-104’, ‘02-17’, and ‘01-202’ were the top five ranked clones at the end of the first rotation, while clones ‘02-17’, ‘Be’, ‘276’, ‘599’, and ‘136’ were the top five ranked clones at the end of the second rotation. Clones ‘02-17’ and ‘599’ demonstrated stable, rapid lateral growth over the two triennial rotations. The tree heights of clones ‘02-17’ and ‘599’ were also highest. Clones ‘N179’ and ‘110’ showed low tree heights and thin basal diameters over the two triennial rotations.
Clone influence on sprout number was significant both in the first and second rotation cycles. The sprout number at the end of the first growth cycle showed an average of 4.61, while the sprout number at the second rotation was 2.06 (Figure S1). Clones ‘N179’, ‘110’, and ‘03-332’ showed relatively higher sprout numbers than most of the other clones, especially at the end of the first rotation. However, clone ‘599’ showed a limited capacity to generate a high number of sprouts after coppice in comparison to other clones over the two rotation cycles. Sprout numbers at the end of the first rotation cycle reached values two times higher than those in the second rotation (Figure S2). The difference in sprout numbers had a strong influence on the height and basal ground cover between the first and second triennial cycles, as the height and basal ground cover were significantly higher at the end of the second rotation cycle than the first rotation cycle. However, the difference in sprout numbers had little effect on the height and basal diameter of the main sprout (Figure S2).

3.3 | Aboveground biomass

At the end of the first rotation, the mean aboveground biomass accumulation per plot differed significantly among clones (Figure 2c), with clone ‘05-33’ producing the lowest biomass of 5.99 t ha\(^{-1}\) year\(^{-1}\) and clone ‘03-332’ producing the highest biomass of 16.33 t ha\(^{-1}\) year\(^{-1}\). Clonal differences
in aboveground biomass per plot at the end of the second rotation were also considerable, ranging from 9.31 t ha⁻¹ year⁻¹ for clone ‘04-107’ to 18.27 t ha⁻¹ year⁻¹ for clone ‘01-30’ (Figure 2c). The biomass at the end of the second rotation cycle was an average of 14.59 t ha⁻¹ year⁻¹, 42% higher than the value from the first rotation cycle.

The clonal ranking of biomass at the end of the first rotation differed from that at the second rotation. Clones ‘03-332’, ‘110’, ‘910-95’, ‘N179’, and ‘01-104’ were the top five ranked clones for the first rotation cycle, while clones ‘50’, ‘599’, ‘Be’, ‘36’, and ‘01-30’ were the top five ranked clones for the second rotation cycle. No clone demonstrated stably high biomass over the two triennial rotations. However, the biomass of the top five ranked clones at the first rotation was low in the second rotation, as the biomass of clone ‘110’ was higher only than clones ‘04-107’ and ‘313’ but lower than most clones at the second rotation. Thus, the clones appeared to produce more biomass in the first rotation and did not perform well in the second rotation. Clones ‘Be’ and ‘36’ were the relatively stable clones over the two triennial rotations (Table S2).

3.4 | Heat value

The heat values showed differences among the 27 clones (Figure 3). The heat values of clones ‘01-30’ and ‘599’ were relatively low, with values of 18.30 and 18.33 MJ, respectively. The top five ranked clones for heat value were clones ‘65’, ‘04-107’, ‘2001’, ‘01-202’, and ‘50’, and the highest value was 19.26 MJ in clone ‘65’.

3.5 | Cell wall composition

Cell wall composition confers the chemical differences of the biomass, with a direct impact on the degradation procedure of the energy conversion process. Understanding specific cell wall chemical features will help understand the chemical properties of the biomass and thus better use renewable energy sources. At the end of the first rotation, we collected the stem of each Populus clone and performed cell wall composition analysis. As shown in Figure 4, the lignin content ranged from 22.4% (clone ‘05-33’) to 28.9% (clone ‘276’), with an average value of 25.0%. The cellulose content of the 27 clones also demonstrated obvious differences, with an average content of 442.99 μg/mg AIRs, ranging from 393.83 (clone ‘599’) to 497.48 (clone ‘2001’) μg/mg AIRs. The relative abundance of neutral sugars showed significant differences between clones. The clearest difference existed in arabinose (Ara), since the content of clone ‘G2’ (9.17 μg/mg AIRs) was more than two times higher than that of clone ‘02-17’ (3.99 μg/mg AIRs). For rhamnose (Rha), fructose (Fuc), mannose (Man), galactose (Gal) and glucose (Glc), the difference between clones could also be higher than 15%. Even for xylose (Xyl), a component of the smallest difference, the value ranged from 258.17 (clone ‘313’) to 305.81 (clone ‘65’) μg/mg AIRs.

3.6 | Correlation analysis of the phenotypes

To explore the relationships between these growth and chemical parameters, we performed a correlation analysis using the data obtained in the above analyses. Noticeably, the height and diameter showed highly positive correlations in both rotations, with Pearson correlation coefficients (PCCs) of 0.8 and 0.82 (p < .05) in the first and second rotations, respectively (Figure 5). In the second rotation, biomass was positively correlated with not only height and diameter but also Xyl content. Among the cell wall components, Rha, Gal and Fuc showed a highly positive correlation with the PCC ranging from 0.64 to 0.83 (p < .05). Both Gal and Fuc were negatively correlated with Man, whereas Rha and Ara were negatively correlated with cellulose (p < .05).

3.7 | Cluster analysis

A cluster analysis based on height, basal diameter, biomass, heat value, cellulose content and survival rate revealed five distinct growth potential classes (Figure 6). Cluster 1 (including clones ‘162’, ‘910-95’, ‘01-104’, ‘313’, ‘02-10’, ‘01-275’, ‘276’, ‘01-202’, ‘04-107’, ‘65’, and ‘02-17’) had excellent growth and heat value; cluster 2 (including clones ‘50’, ‘Be’, ‘136’, ‘01-243’, ‘2001’, ‘03-176’, ‘04-95’, ‘01-30’, and ‘110’) exhibited high cellulose content; cluster 3 (including clones ‘04-143’, ‘G2’, ‘05-33’, and ‘N179’) showed a high survival rate; cluster 4 (including clone ‘03-332’) exhibited high biomass; and cluster 5 (including clones ‘36’ and ‘599’) exhibited high growth.
FIGURE 4  Lignin, cellulose and monosaccharide composition of cell wall material isolated from the 27 hybrid *Populus* clones. Contents of (a) lignin; (b) cellulose; (c) Rha, rhamnose; (d) Fuc, fucose; (e) Ara, arabinose; (f) Xyl, xylose; (g) Man, mannose; (h) Gal, galactose; and (i) Glc, glucose. The gray line in each panel represents the mean of the 27 clones.

FIGURE 5  Correlation heatmap of phenotypic data. Red and blue squares represent positive and negative correlations, respectively. Asterisks indicate significance at $p < .05$. 
3.8 | Expression pattern of key regulatory genes

To explore the potential molecular mechanisms controlling the growth and cell wall phenotypes among these *Populus* clones, we selected 20 key regulatory genes and analyzed their expression patterns in the developing xylem of 12 selected clones that covered different clusters and different species. The 20 regulatory genes included eight MYB family TFs (*MYB3*, *MYB4*, *MYB7*, *MYB17*, *MYB27*, *MYB46*, *MYB63*, and *MYB83*), five NAC family TFs (*NST1a*, *NST1b*, *NST1c*, *NST1d*, and *VNI2*), two other TFs (*WOX4* and *WRKY6*) and five cell wall–related structural genes (*BGAL10*, *CSLC5*, *GH9C2*, *UGT74F1*, and *XTH5*).

As shown in Figure 7, the expression patterns of the 20 selected genes varied among the *Populus* clones. For instance, *BGAL10* showed the highest expression in clone ‘N179’ and the lowest expression in clone ‘G2’. Four *NST1* homologs (*NST1a*, *NST1b*, *NST1c*, and *NST1d*) and two master switches (*MYB46* and *MYB83*) in the first layer of transcriptional regulatory network in the secondary cell wall formation (Zhang, Xie, et al., 2018) showed similar expression patterns, with the highest expression level occurring in clone ‘36’ among the detected clones. In addition, other TFs and functional genes involved in secondary cell wall formation, such as *MYB63*, *WOX4* and *XTH5*, also showed the highest expression level in clone ‘36’. In contrast, the transcriptional repressor *MYB4* showed distinct patterns with the lowest expression level in clone ‘36’ and the highest expression level in clone ‘G2’. Moreover, *MYB17*, *MYB27*, *MYB3*, *WRKY6* and *UGT74F1* were highly expressed in clone ‘N179’.

3.9 | Integrated coexpression network of key regulatory genes

To further expand the understanding of the molecular mechanisms of the selected key regulatory genes, we constructed an integrated coexpression network based on the expression correlation among these regulatory genes and their coexpressed genes from the Phytozome database. As shown in Figure 8, the top-layer TFs of the transcriptional regulatory network of secondary cell wall biosynthesis, such as *NST1s*, *MYB46/83*, and *MYB63*, were strongly coexpressed with each other and with some cell wall–related genes (Table S3).

Moreover, we performed a correlation analysis to test the relationship between the expression of key genes and the phenotypic data. As shown in Figure 9, several secondary cell wall biosynthetic markers, such as *NST1c*, *MYB46/83/63*, *VNI2*, and *WOX4*, were positively correlated with the lignin content, which indicates the reliability of the correlation analysis. *BGAL10* and *CSLC5* were negatively correlated with Rha and Gal, whereas they positively correlated with different cell wall components—*BGAL10* for Xyl and *CSLC5* for Man. *MYB63* and *WOX4* were positively correlated with height and diameter in the first rotation, but *NST1c*, *MYB46*, and *XTH5* were strongly correlated with height rather than with diameter in the same rotation. *MYB27* showed a strong correlation with multiple phenotypes, positively correlated with biomass in the first rotation and cell wall compositions Man and Glc, and negatively correlated with Fuc and Gal.

4 | DISCUSSION

To date, an evaluation of the poplar clones suitable for SRC in northern China has been conducted (Gong et al., 2011; Guo & Zhang, 2010), and several studies have revealed the genetic basis of poplar growth (Yáñez et al., 2019; Zhang, Zhan, et al., 2018; Zhang et al., 2017). Based on these studies, the clonal differences in ecophysiological and morphological characteristics were identified in several poplar clones. However, knowledge of the agronomic performance of fast-growing poplar clones and the molecular mechanisms of growth phenotypes in middle China remains limited. In this study, we mainly focused on the native, fast-growing *Populus* in China and evaluated their agronomic performance in Henan, China. In addition, we analyzed the expression profiles of the key regulatory genes and integrated the coexpression network to explore the potential relationships between the regulatory genes and phenotypes.

As one of the parents of the 10 clones in this study (Table 1), female clone ‘Danhong’ has fast growth with
DBH (diameter at breast height) of 4–8 cm per year. It resists infection by the pest *Apriona germari* (Hope) with the susceptible rate below 20% (Zhao et al., 2008). Male clone ‘Nanyang’ (clone ‘276’ in this study), which is one of the parents of the six clones (Table 1), shows great growth performance in DBH, height, and volume. It has high resistance to *Anoplophora glaberipennis*, moderate resistance to *Apriona germari* and moderate tolerance to waterlogging (Hu et al., 2013). Clone ‘Beiyang’ is the male parent of six clones (Table 1), and it also shows fast-growing and strong
resistance to *Anoplophora glaberipennis* and soil barren tolerance (Cao et al., 2016). The outstanding performance of these parents provides a solid foundation for the selection of elite variety. However, the characteristics of productivity, adaptability and pest resistance of these clones need to be further studied.

Selecting clones suitable for SRC was important for biomass yields and quality control. Many factors could influence final biomass yields; the internal factors include survival rate, disease resistance and biomass productivity, while the external factors include climate, irrigation and fertilization. Among the factors, survival rate is the first important index for clone selection in the rooting stage. Clones in our study exhibited a wide range of survival rates, from 29.17% to 81.25%, which is common when evaluating a large number of clones. In the last 2 years of the second rotation (2012 and 2013), the rainfall in May–August was lower than that in previous years, and the annual accumulated temperature of ≥10°C was higher than that in previous years (Figure S1), which might be the reason for the low survival rates in the second two rotations. Most clones showed a survival rate >50%, while clones ‘01-243’, ‘N179’, and ‘04-143’ even displayed survival rates higher than 75% (Figure 1b). However, clones ‘36’, ‘02-10’, ‘313’, ‘599’, and ‘Be’ experienced survival rates lower than 45%. Considering that the survival rate of the three clones declined after winter, their low survival rates might be explained by cold and dry winter season in Henan, China. The three clones could not tolerate the climate, and most of the seedlings died in winter.

Growth vigor-related traits such as tree height and basal diameter were the other important selection criteria for SRC biomass yields. In our study, the ranking of tree height and basal diameter changed from the end of the first triennial rotation to the end of the second triennial rotation. Plant productivity is influenced by genetic factors and many other factors, such as soil, growth of weeds and climate factors. The reduced summer rainfall and increased annual accumulated temperature in the last 2 years of the second rotation might have been the reason for the growth differences in the two rotations. In addition, the improved growth in the second rotation might also have been related to the change in space and light conditions after completion of the first coppice. This finding was consistent with those of previous studies. The shift in clonal ranking might have been caused by the difference in cutting. As reported previously, coppicing strongly promotes the production of new sprouts (Paris et al., 2011).

Biomass production is another important index for clone selection. The biomass data reported here for the 27 poplar clones differed considerably (Figure 2). Zhao and Zhou (2005) reported that the net primary productivity of *Populus* is 10.14 ± 5.61 t ha⁻¹ year⁻¹ in China. For most of the poplar clones, the biomass was higher than 10 t ha⁻¹ year⁻¹. The dry matter production of clones ‘36’ and ‘01-30’ was ~18 t ha⁻¹ year⁻¹, showing great performance at our field site. However, clones ‘04-107’, ‘313’, and ‘110’ showed much lower productivity, less than 10 t ha⁻¹ year⁻¹. Cannell proposed that the annual maximum yield was 10–12 t ha⁻¹ year⁻¹ (Cannell, 1989), but yield can be influenced by many factors. Our results demonstrated that at least 24 clones could be an economically feasible option in test area because of their high biomass production. Under high stand density, the rotation age of the hybrid poplar could be as short as 3–4 years (Kauter, Lewandowski, & Claupein, 2003). In our study, the production biomass of most of the poplar clones exceeded the proposed annual maximum yields, which indicates that a 3-year rotation is suitable in Henan, China.
Combustion is the most common way of converting biomass fuels to energy (Guo & Zhang, 2010; Parikk, 2004; Paris et al., 2011). The heating value, moisture content and elemental composition of biomass have a significant effect on the biomass combustion process (Deckmyn et al., 2004). The heating value calculated here for the 27 poplar clones ranged from 18.30 to 19.26 MJ (Figure 3), similar to the previously reported value (Cuiping, Chuangzhi, Yanyongjie, & Haitao, 2004). In addition, the top five ranked clones based on heat value were clones ‘65’, ‘04-107’, ‘2001’, ‘01-202’, and ‘50’, suggesting that these clones may have the potential for direct combustion for bioenergy production.

Cellulose, hemicellulose and lignin are the major components of woody plant biomass. A cell wall chemical analysis that determines the contents of cellulose, hemicellulose and lignin is sometimes conducted to characterize the quality of biomass. Biomass has been used to produce heat and electricity, but recently, considering the economic benefits and environmental protection, biomass has become attractive for producing renewable biofuels (e.g., bioethanol; Ragauskas et al., 2006). In previous studies, researchers have found that chemical compositions of poplar wood depend on not only species, clones, age and tissue type (Guidi et al., 2009; Kauter et al., 2003; Klasnja, Koptovic, & Orlovic, 2003; Tharakan, Volk, Abrahamson, & White, 2003; Willför, Sundberg, Pranovich, & Holmbom, 2005) but also nitrogen supply, location and even rotation cycle (Guidi et al., 2009; Kaakinen, Saranpää, & Vapaavuori, 2006; Pitre, Cooke, & Mackay, 2007). We found that the relative abundance of neutral sugars and cellulose showed significant differences between clones, similar to previous findings (Macaya-Sanz et al., 2017). From a biochemical conversion perspective, our data suggested that different poplar clones would lead to a diverse biochemical conversion yield. Ethanol is obtained from carbohydrates through hydrolysis and fermentation (Pauly & Keegstra, 2010). For woody plants, cellulose is the major carbohydrate for a large proportion of wood. Since ethanol is chiefly obtained through cellulose fermentation (Butner, Elliott, Sealock, & Pyne, 1988), clone ‘2001’ with high cellulose content would ensure maximum yields, suggesting a good candidate for biofuel SRC poplar clones. Except for cellulose content, the fermentability of the released monosaccharides was another issue that needs to be considered in biofuel conversion since the current ethanol fermentation process utilizes hexoses such as glucose and mannose with a better efficiency than xylose, arabinose, and pentoses (Pauly & Keegstra, 2010). Therefore, clones with high contents of xylose, arabinose and pentoses may not be suitable for biofuel applications.

Selection of superior genetic material with high biomass production is critical for commercial SRC plantations. Since growth, biomass, heat value and chemical composition are all very important for selection, a hierarchical cluster analysis was an effective procedure for grouping clones considering all these selection criteria (Guo & Zhang, 2010). A cluster analysis based on height, basal diameter, biomass, heat value, cellulose content and survival rate revealed five distinct growth potential classes (Figure 3). Cluster 4 (including clone ‘03-332’) exhibited high biomass, and cluster 5 (including clones ‘36’ and ‘599’) exhibited high growth. These clones had the highest potential to serve as excellent biomass producers in this region. Cluster 1 (including clones ‘162’, ‘910-95’, ‘01-104’, ‘313’, ‘02-10’, ‘01-275’, ‘276’, ‘01-202’, ‘04-107’, ‘65’, and ‘02-17’) had excellent growth and heat values. Although they did not perform as well as the clones in cluster 5, they will be good biomass producers. Cluster 2 (including clones ‘50’, ‘Be’, ‘136’, ‘01-243’, ‘2001’, ‘03-176’, ‘04-95’, ‘01-30’, and ‘110’) exhibited high cellulose content. These clones may have the potential to provide various wood production services in this region. Cluster 3 (including clones ‘04-143’, ‘G2’, ‘05-33’, and ‘N179’) showed a high survival rate.

As the majority of the biomass of trees, secondary cell walls are synthesized under the control of a complex regulatory network with coherent feedforward and feedback loops (Zhang, Xie, et al., 2018). In Arabidopsis, secondary cell wall biosynthesis is regulated by a series of transcriptional activators and repressors (Zhao & Dixon, 2011). Brown, Zeef, Ellis, Goodacre, and Turner (2005) using reverse genetics and gene expression profiling identified novel genes that involved in Arabidopsis secondary cell wall formation. Guo et al. (2014) using a combined approach of coexpression and cell wall metabolomics obtained certain coexpressed gene modules that positively correlated with distinct cell wall characteristics in rice. Unlike Arabidopsis and grasses, perennial woody species display strong secondary growth. We found that the expression patterns of positive regulators of cell wall biosynthesis (NST1s, MYB46, MYB83, MYB63, and WOX4) showed an opposite pattern with the transcriptional repressor MYB4 (Figure 7). These TFs are well-known key regulators that directly control secondary cell wall biosynthesis (Zhao et al., 2011; Zhang et al., 2018). Noticeably, clone ‘36’ showed great biomass performance among the 27 analyzed clones (Figure 2), it had high expression levels of those positive regulators. This result indicates that the master regulators in the top layers of the transcriptional regulatory network play a dominant role in poplar biomass production. A large number of cell wall-related genes, such as IRXs, LACs, FLAs, and AGPs, were strongly coexpressed with these regulatory genes in the integrated coexpression network. This result further suggests that these regulatory genes play important roles in cell wall-related processes.

Our research was initiated with the objective of quantifying the biomass yield of 27 poplar clones and evaluating...
their performance in Henan, China. The most productive clones were ‘36’ and ‘01-30’. At the end of the second triennial rotation, the maximum aboveground biomass production reached 18 Mg ha\(^{-1}\) year\(^{-1}\). However, a significant difference in biomass production among clones was evident. A cluster analysis based on height, basal diameter, biomass, heat value, cellulose content and survival rate revealed five growth potential classes. Clones ‘03-332’, ‘36’, and ‘599’ exhibited high biomass and growth and had the greatest potential to serve as excellent biomass producers in Henan, China. The expression of key genes and the integrated coexpression network provide the molecular mechanisms of poplar biomass performance.

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CONFLICT OF INTEREST
The authors declare that they have no competing interests.

AUTHORS’ CONTRIBUTIONS
J.Z. and J.H. conceived and designed the study. J.Z. and X.S. (Song) analyzed data and wrote the manuscript. L.Z., H.J., Z.Z., X.S. (Su), X.L., and J.H. performed experiments. M.L. (Song) analyzed data and wrote the manuscript. L.Z., H.J., and J.H. contributed to discussion and manuscript revision. All the authors were involved in the discussion of the data and approved the final manuscript.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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