Due to the growing demand for transportation fuels and concerns about the greenhouse gas emissions derived from the use of fossil fuels, the development of alternative fuels from renewable resources, such as lignocellulosic biomass, is of paramount importance. This is compounded by the fact that there are increasing pressures and limitation on available arable land for renewable biomass production, and therefore, how to obtain more biomass resources and how to make full use of these biomass resources are key issues. Poplar (Populus spp.) is one of the fastest-growing temperate trees in the world and is a very promising raw material for the production of biofuels and other bio-based commodities. As the first tree species to have its genome sequenced, and significant continuing efforts towards resequencing of different species/varieties, poplar resources will undoubtedly pave the way for the targeted cultivation of new poplar varieties suitable for biofuel production. In this article, we summarized that the main problems faced by using poplar as a biomass resource for biofuel production are the inherent recalcitrance of lignocellulosic biomass, and highlighted the response status on improving the biomass yield and efforts towards developing efficient poplar varieties for biofuel production.

**KEYWORDS**
bioenergy, biofuel production, biomass yield, efficient utilization, genetic modification, poplar
cannot provide the needed sustainable energy demands, especially with increasing global populations. Lignocellulosic crops including Miscanthus (Wang et al., 2020), switchgrass (Cooney et al., 2017) and fast-growing trees, which are rich in cellulose, hemicelluloses, and lignin, can be converted to generate heat, electricity, biogas, and ethanol. They are commonly referred to as second-generation bioenergy crops that are potential alternatives for fossil fuel. The plant cell walls of lignocellulosic biomass provide structural support for growth and act as barriers to pathogen invasion. Cellulose is the main component of plant cell walls and it is the most abundant biopolymer on earth. Through biocatalytic processes, cellulose can be converted into bioenergy such as bioethanol and biomethane, and other bio-based products. It is estimated that biomass energy fixed by plants each year is 5–10 times the worldwide annual energy consumption (Zhang et al., 2018).

Previous studies have shown that trees are essential natural resources for sustainable and renewable energy production; however, they also provide additionally important ecological and societal values (Ragauskas et al., 2014; Zhang et al., 2018).

As one of the fast-growing bioenergy crops, poplar is rich in carbohydrates (cellulose and hemicellulose), which are promising resource for renewable biofuels and bioproducts and can be a sustainable alternative to petroleum-based products (Ragauskas et al., 2006). Compared with other bioenergy crops, poplar is perennial and displays rapid growth and high productivity, and do not require a large amount of annual energy and financial inputs (tillage and planting, fertilizer, herbicides, and pesticides; Littlewood et al., 2014). Ecologically, poplars are very helpful in restoring disturbed environments, controlling land desertification, gradually reducing arid areas, continuously increasing soil fertility, and improving the ecological environment affected by anthropogenic activities (Bilek et al., 2020). In addition, the genomes of several poplars have been sequenced (Yang et al., 2019) that facilitates experiments for concise genetic modification for higher biomass yield (Han et al., 2020) and/or unique cell wall traits (Mahon & Mansfield, 2019). For example, research on the synthesis and deposition of plant cell walls and the genetic mechanisms contributing to formation and regulation of fibrous biomass has been identified, and new varieties of biomass plants that can be efficiently transformed via biochemical conversion process could be developed and cultivated. Porth and El- Kassaby (2015) clearly showed that poplars have significant inherent variability in cell wall characteristics, and a good cellulose and lignin ration, which can be served as a good feedstock as bioethanol crops. However, lignocellulosic biomass, such as poplar, display recalcitrance to microbial and enzymatic hydrolysis due to its higher lignin content. Pretreatment prior to biochemical conversion of lignocellulosic biomass is considered to be an effective method limiting its inherent recalcitrance. However, many obstacles remain to be overcome in biomass pretreatment and biochemical conversion in terms of cost and energy efficiency, and environmental friendliness and yield maximization (Al- Ahmad, 2018).

Understanding the intricacies of the biosynthesis and regulation of plant cell formation walls is critical to the genetic modification of plant raw materials, which will allow the efficient conversion and cost-effective production of cost-competitive biofuels and bio-based products. Therefore, poplars are promising feedstocks for renewable biofuel production and could play a vital role in renewable biofuel production.

2 | BARRIERS FOR USING POPLAR AS BIOMASS FEEDSTOCKS FOR BIOFUEL AND BIOENERGY PRODUCTION

2.1 | Biomass yield

A high-yielding woody species managed in a coppice system is described as short-rotation coppice (SRC). Currently, poplar (Populus spp.) and willow (Salix spp.) are the priority SRC, and are harvested on a 3- to 5-year rotation. However, field measured yield is variable among species and genotypes and is affected by environmental factors and climatic conditions. Rae et al. (2004) measured the yield of SRC poplar in an F2 progeny of interspecific pedigree derived from Populus trichocarpa “Torr. & A. Gray” × P. deltoides “Marsh” grown in the United Kingdom and found the biomass yield after the first coppice ranged from 0.04 to 23.68 oven-dry tons (odt) ha−1 year−1. Aylott et al. (2008) performed a large study consisting of 49 sites for SRC poplar with 16 genotypes in the United Kingdom, and found that the average yield over the two rotations was 6.3 odt ha−1 year−1, which is lower than willow genotypes (9.0 odt ha−1 year−1). Naso I Di Nasso et al. (2010) compared the performance differences of SRC poplar (P. deltoides Bartr. clone “Lux”) under different harvest regimes in a long-term (12 years) field trial in southern Europe, and the results showed that the annual biomass yield was 9.9, 13.8, and 16.4 odt ha−1 year−1 for annual, biennial, and triennial cycles, respectively. Dillen et al. (2013) reported the aboveground biomass yield and energy balance of 17 poplar clones with SRC on different growth site—on degraded land, the highest yield of different clones during the fourth round of rotation reach 10.5 odt ha−1 year−1, whereas the average yield was only 4.3±3.4 odt ha−1 year−1. Verlinden et al. (2015) compared aboveground biomass productivity of 12 poplar genotypes during two biennial rotations in Belgium and found two hybrid poplar genotypes (P. deltoides × P. nigra clone “Hees” and P. trichocarpa × P. maximowiczii clone “Skado”) had the highest productivity (16 odt ha−1 year−1 in
the second rotation). Zhang, Song, et al. (2019) evaluated the agronomic performance of 27 hybrid poplar clones over two coppice triennial rotations in northern China, and showed that the aboveground biomass of the 27 poplar clones ranged from 5.99 to 16.33 odt ha\(^{-1}\) year\(^{-1}\) for the first triennial rotation and from 9.31 to 18.27 odt ha\(^{-1}\) year\(^{-1}\) at the end of the first and second triennial rotations, respectively. In addition, other physiological or cultivation conditions can affect biomass accumulation. For example, poplar clones with small stomata, large leaf area, and high dry mass ratio produce higher biomass under drought stress (Niemczyk et al., 2019). Intercropping hybrid poplar and switchgrass (*Panicum virgatum* L.) can also improve land use efficiency for biomass production (Kimura et al., 2018).

Thus, it is fair to conclude that biomass yield is significantly affected by genetic variation, local environment, and climatic condition to mention only a few potential factors. “How to select a genotype with stable above-average performance in a local environment?” is a big challenge for researchers and breeders.

### 2.2 Biomass recalcitrance

Lignocellulosic biomass is an intricate matrix of carbohydrate polymers (primarily cellulose and hemicellulose) and aromatic polymer lignin (Stefanidis et al., 2014). To convert lignocellulosic biomass into renewable biofuels and biomaterials, high molecular weight polymers stored in the plant cell walls need to be deconstructed and liberated as monosaccharide constituents. However, the high costs associated with biomass pretreatment and saccharification are the key barriers that affect industrial commercialization of these second-generation crops for biofuel production (DeMartini et al., 2013).

Biomass recalcitrance is associated with many natural factors, such as particle size, biomass porosity, lignin content, the ratio of syringyl and guaiacyl units (S/G), lignin structure, cellulose degree of polymerization (DP) and crystallinity, cellulose accessibility, and hemicellulose content (Himmel et al., 2007; Mansfield et al., 1999). For instance, the hydrolysis of the amorphous cellulose is faster than that of the crystalline cellulose, which is due to limiting access of enzyme toward by strong inter- and intra-chain hydrogen-bond crystalline core of cellulose (Zhang & Lynd, 2004). Significant research over the years has focused on correlating substrate features to biomass recalcitrance. However, many of the published findings are conflicting. For example, high lignin content is considered to limit the rate of enzymatic hydrolysis of biomass because it physically blocks the accessibility of the enzymes to cellulose and adsorbs cellulase irreversibly (Meng & Ragauskas, 2014; Mooney et al., 1998). In contrast, Studer et al. (2011) studied a large natural poplar population and reported that no significant correlation was observed between lignin content and enzymatic hydrolysis, whereas Mansfield et al. (2012) clearly showed that poplar trees engineered to have higher S/G ratios preformed significantly better during bioconversion to ethanol evaluation using both steam explosion and organosolv pretreatments. Similarly, a lower cellulose DP was reported to improve enzymatic hydrolysis due to the increased cellulose reactivity and numbers of cellulose chain reducing ends (Hallac & Ragauskas, 2011). However, Sinitsyn et al. (1991) indicated that reducing the DP of cotton linters by Gamma-irradiation had no significant impact on the hydrolysis rate. The inconsistencies in these studies might be attributed to the complex interactive effects of all the structural factors, and that no single consistent evaluation criteria were used in the abovementioned studies.

### 3 STRATEGIES FOR IMPROVING BIOFUEL AND BIOENERGY PRODUCTION FROM POPLAR

#### 3.1 Biomass pretreatment for enhanced bioenergy production

Due to its high recalcitrance, lignocellulosic biomass needs one or more pretreatment processes to help increase accessible surface area before their effective conversion into liquid biofuels. Pretreatment methods include physical, chemical, biological, and their combination thereof (Singh et al., 2015). Chemical pretreatment is the most studied strategy to reduce the lignification of lignocellulosic biomass for bioethanol production (Anwar et al., 2014). However, some fermentation inhibitors (e.g., furfural, hydroxymethylfurfural (HMF), and acetic acid) are generated and accumulated when using concentrated acids regents under high temperature and pressure conditions (Camargo et al., 2019; Keating et al., 2006). Lignin content has a major influence on cellulose ultrastructural and can impact changes to the other cell wall constituents during pretreatment. The dilute acid pretreatment (DAP), for example, significantly affects cellulose ultrastructure, such as cellulose crystallinity index (CrI) and DP. Sun et al. (2014) compared the structural changes of cellulose in untreated and partially delignified poplar (*P. trichocarpa × deltoids*) after dilute sulfuric acid pretreatment, and found that decreased lignin content during DAP could promote an increase in the size of the cellulose microfiber as well crystallite size. Meng et al. (2016) compared the effects of DAP and alkaline pretreatment on the chemical compositions of poplar substrates, and found that xylan was almost completely removed after DAP, but only ~37% was removed after alkaline pretreatment. In contrast to most acid and alkaline pretreatments, ammonia fiber expansion (APEX) pretreatment has
the advantage that the resultant biomass is ready for hydrolytic depolymerization and fermentation without the need of detoxification or external nutrient supplementation (Lau & Dale, 2009). Balan et al. (2009) reported that under the optimal AFEX pretreatment conditions (an ammonia to biomass ratio of 2:1 at 180°C for 30 min), the conversion efficiency of glucon and xylan in *P. nigra × maximowiczii* was as high as 93% and 65%, respectively (Table 1). Liquid hot water (LHW) pretreatment is an effective thermochemical approach to deconstruct plant cell walls for solubilized sugar fermentation with little or no inhibitors generated (Negro et al., 2003). The highest fermentable sugar yield with minimal formation of sugar decomposition products from LHW pretreated poplar was obtained under pretreatment conditions optimized to 200°C for 10 min (Kim et al., 2009). Another hydrothermal pretreatment, steam explosion, can be affected by residence time, temperature, moisture content, and chip size of the selected substrate (Cullis et al., 2004). The optimized condition for steam explosion of poplar was suggested to be 210°C for 4 min, which resulted in ~95% cellulose recovery and ~60% enzymatic saccharification yield (Negro et al., 2003).

### TABLE 1 Pretreatment for poplar biomass and its effects on saccharification

| Pretreatment    | Populus Species     | Detailed pretreatment                                              | Effect on saccharification                                                                 | Reference       |
|-----------------|---------------------|---------------------------------------------------------------------|------------------------------------------------------------------------------------------|-----------------|
| DAP             | *Populus trichocarpa × deltoides* | Slightly higher glucose yield during 48 h. Xylan was almost completely removed. |                                                                      | Meng et al. (2016) |
| Alkaline        | *P. trichocarpa × deltoides*      | ~37% of xylan was removed.                                           |                                                                       | Meng et al. (2016) |
| LHW             | *P. nigra*                | 230–240°C                                                          | Maximum enzymatic hydrolysis yield ~70%.                                                   | Negro et al. (2003) |
| LHW             | Hybrid poplar          | 200°C, 10 min                                                       | Highest fermentable sugar yield with minimal formation of sugar decomposition products.     | Kim et al. (2009) |
| Steam explosion | *P. nigra*               | 210°C, 4 min                                                        | Cellulose recovery ~95%, enzymatic hydrolysis yield ~60%, simultaneous saccharification and fermentation yield of 60% of theoretical, xylose recovery ~41%. | Negro et al. (2003) |
| AFEX            | *P. nigra × maximowiczii* | 180°C, 2:1 ammonia to biomass loading, 23% moisture, 30 min        | High glucan and xylan conversion (93% and 65%, respectively).                                | Balan et al. (2009) |
| ILs             | Poplar                 | [Hpy]Cl, [Hnim]Cl, or [Hmim]Cl; 90°C or 100°C for 30 min            | High yields of cellulose-rich materials from [Hnim]Cl treated under 90°C for 30 min.        | Wang et al. (2017) |
| DES             | Triploid of *P. tomentosa* Carr. | Choline chloride–lactic acid (ChCl-LA) and choline chloride–oxalic acid (ChCl-OA) at 80–120°C for 6 h | The yield of the regenerated lignin ranged 44%–75% after DEG pretreatment. The depolymerization of lignin fractions was the dominant reaction during DES pretreatment. | Hong et al. (2020) |

Abbreviations: AFEX, ammonia fiber expansion; DAP, dilute acid pretreatment; DES, deep eutectic solvent; ILs, ionic liquids; LHW, liquid hot water pretreatment.
biodiesel refining due to their convenient synthesis, wide availability from cheap components, recyclability, and bio-degradability. DESs are novel and green solvents that can extract high-purity lignin from lignocellulose in relatively high yield. After DES pretreatment of choline chloride-lactic acid (ChCl-LA) and choline chloride-oxalic acid (ChCL-OA) at 80–120°C for 6 h, the yield of the regenerated lignin from poplar ranged from 44% to 75% (Hong et al., 2020). This latter approach provides a viable way to produce value-added chemicals from lignin.

3.2 Genetically improving poplar as feedstocks for biofuel production

The secondary cell walls (SCW) accounts for a main component of the woody plant biomass. SCWs are composed of lignin, cellulose, and hemicellulose. To date, it has been shown that polymeric lignin is the primary factor affecting biomass recalcitrance and limiting the application of lignocellulose in economically viable bioenergy production of second-generation feedstocks. Lignin is mainly composed of p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) monolignols that are synthesized via the phenylpropanoid pathway (Raes et al., 2003). Regulation and expression of lignin biosynthetic genes are associated with several transcription factors (TF). Zhang et al. (2018) presented three layers of the regulatory network of transcription factors in SCW formation. In the first layer, a group of closely related NAC (NAM, ATAF, and CUC) act as master switches, activating the expression of downstream TFs or structural genes related to SCW development (Hu et al., 2010; Liu et al., 2015; Zhong & Ye, 2015). MYB46 and MYB83 as the master switches at the second layer which are directly regulated by SND1 (Zhong et al., 2007). In the third layer, most of the regulation of SCW biosynthesis TFs belong to the MYB gene family. Therefore, understanding the regulatory mechanism of SCW biosynthesis is critical for improving biomass to be more amenable for enzymatic hydrolysis.

Transgenic technologies are an efficient approach to obtain genetically manipulated plants. Several studies have proven that lignin modification in poplar can facilitate improved saccharification for biofuel production. Wang et al. (2012) compared the saccharification efficiency of knock-down (antisense) transgenic poplar (P. tomentosa) lines of two monolignol biosynthetic genes 4CL or CCoAOMT and showed that the 4CL or CCoAOMT knock-down lines displayed up to 10% reduction of total lignin content, with no changes in growth rate and biomass yield. Moreover, increased saccharification was observed in the CCoAOMT knock-down lines, but not in the 4CL knock-down lines. CCR is the enzyme catalyzing the first step in the monolignol-specific branch of the lignin biosynthetic pathway. Knock-down of CCR in hybrid poplar (P. tremula × alba) significantly enhanced the saccharification under different pretreatments (e.g., none, two alkaline, and one acid pretreatment) and had up to 161% increased ethanol yield (Van Acker et al., 2014). Gui et al. (2019) identified a lignin biosynthesis-associated transcription factor (LTF) from Populus, which directly binds the promoter of 4CL. Mutation of LTF1 in its unphosphorylated state inhibits the lignin deposition in developing xylem cells. When LTF1 is phosphorylated by PdMPK6 in response to external stimuli (such as wounding), it will be degraded through the proteasome pathway, resulting in activation of lignification. Gui et al. (2020) and Cao et al. (2020) improved poplar biomass quality using fiber-specific suppression (F-LS) of lignin biosynthesis, in which lines showed normal lignin deposition but reduced lignin content in fiber cell walls and 67% increasing in glucose saccharification. Recently, gene editing has also been successfully used in poplars (An et al., 2020; Zhou et al., 2015). CRISPR-knock out of poplar 4CL in poplar resulted in a decrease in S/G ratio (Zhou et al., 2015).

In addition to the monolignol biosynthetic genes, genes involved in other cell wall components, such as pectin, have been shown to be good targets for genetic modification to improve sugar release in poplar. Galacturonosyltransferase (GAUT) is an α-1,4-galacturonosyltransferase that synthesizes homogalacturonan (HG) for pectin biosynthesis. Biswal et al. (2015) engineered poplar to modify the expression of GAUT12.1 and found that down-regulation of GAUT12.1 reduced the contents of xylan and pectin during the wood formation process, and decreased the recalcitrance, thus making it easier to extract the cell wall components and increasing the growth of poplars. Repressing the expression of GAUT4 in P. deltoides was also shown to improve its biomass yield and sugar release through reducing HG:GalAT activity and consequently pectin HG and rhamnogalacturonan II content (Biswal et al., 2018). Serine hydroxymethyltransferase 2 (SHMT2) is an enzyme functioning in cellular one-carbon pathways. Overexpression of SHMT2 was recently shown to increase the biomass yield and sugar release, and concurrently decrease the lignin content in transgenic P. deltoides (Zhang, Li, et al., 2019). The results from transgenic poplar demonstrated that genetic modification of cell wall components effectively overcame cell wall recalcitrance and facilitated the conversion to biofuel production.

Moreover, genetic modification of the structure of cell wall components can also affect the efficiency of biomass pretreatment. Expressing the AoxFMT gene in poplar using tissue-specific promoters was shown to introduce novel ester bonds into the lignin in transgenic poplar (P. alba × grandidentata) effectively manifesting in natural “zip-lignin” (Wilkerson et al., 2014). Compared with untransformed poplar, the “zip-lignin” poplar showed higher glucose and xylose yields following both alkaline-only pretreatment (Wilkerson et al., 2014), copper-catalyzed alkaline hydrogen peroxide
pretreatment (Bhalla et al., 2018), and ILs pretreatment (Kim et al., 2017).

3.3 Strategies for discovering novel regulators to improve biofuel production from poplar

Current applications of genetically modified poplar are still largely restricted to use cell wall biosynthetic genes. “How to effectively identify the upstream regulator of cell wall biosynthesis?”—this may be a key issue to improve the genetic modification efficiency to simultaneously manipulate multiple cell wall components (compared to a gene-by-gene approach). Although the regulatory pathway has been identified in several model species such as Arabidopsis and switchgrass, the regulatory pathways in poplar (woody biomass) may differ from that of herbaceous biomass due to its perennial growth nature and strong secondary growth (Zhang et al., 2018, 2020).

Quantitative trait loci (QTLs) are regions within genomes that contain genes associated with a particular quantitative trait. QTL mapping of an inbred F2 population from a cross between P. trichocarpa (clone 93-968, female) and P. deltoides (clone ILL-129, male) identified five robust QTL hotspots that appear to be related to poplar biomass across multiple coppice cycles (Rae et al., 2009). With the development of high-throughput phenotyping, more phenotypes were used for QTL mapping studies. El Malki et al. (2013), using near-infrared spectroscopy, predicted 12 chemical properties and saccharification potential-related phenotypes from 479 P. nigra and detected 10 QTLs located on 6 linkage groups. This provides promising genomic regions to screen gene models for further functional validation.

Genome-wide association analysis (GWAS) is also an effective approach to explore genomic regions linked to complex phenotypic traits. Allwright et al. (2016) performed a GWAS analysis using a 12 K Illumina genotyping array (including 10,331 single nucleotide polymorphisms [SNPs] in 14 QTL regions and 4,648 genes) that obtained from 714 individuals of a European black poplar (P. nigra L.), and identified several novel genes including transcription factor that are associated with biomass yield and other bioenergy traits. Guerra et al. (2019) identified 813 k SNPs from 461 P. trichocarpa clones and performed GWAS of 20 traits. Among these traits, cellulose synthase CesA1A was associated with C6-sugars, whereas Laccase LAC1A and Phenylalanine Ammonia-Lyase PAL5 were significantly associated with lignin S/G ratio (Guerra et al., 2019). Therefore, GWAS may also provide an effective means to discover novel regulatory genes without unexpected results.

4 FUTURE PERSPECTIVES

Populus is a promising eco-friendly and cost-effective energy plant. Plantations established on marginal lands can provide environmentally and economically beneficial treatment for the soil (including both remediation and stabilization benefits) and will not compete with food production. Therefore, coupling bioenergy production with land reclamation can play an important role in the environmental restoration of desertification land and arid land. In the future, forest-derived lignocellulosic feedstocks should be used for the production of biofuels and bio-based materials to create sustainable and ecologically sound resources to displace petroleum-derived fuels and materials.

To improve biofuel and bioenergy production from poplar, increasing biomass yield and reducing biomass recalcitrance are key issues that need to be resolved. From the technical perspective, field management should be strengthened to increase poplar biomass yield, and pretreatment methods should be optimized to reduce the recalcitrance of poplar biomass and increase its conversion efficiency for bioenergy production. From the breeding perspective, genetic modification is an effective targeted method to promote biomass production and modify the cell wall compositions to reduce the recalcitrance of biomass. In addition, with the rapid development of high-throughput technology, significant “big data” have been made publicly available, which could be exploited via advanced data mining, including multi-omics datasets, such as transcriptome, proteome, metabolome datasets, and other large-scale datasets including SNP from GWAS and expression quantitative trait locus (eQTL) study. Finally, combining breeding with advanced engineering solutions could create an ideal poplar for biofuel and bioenergy production.

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DATA AVAILABILITY STATEMENT

There is no data available.

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