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REVIEW

Sweet sorghum and *Miscanthus*: Two potential dedicated bioenergy crops in China

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Abstract

Among the potential non-food energy crops, the sugar-rich C_4 grass sweet sorghum and the biomass-rich are increasingly considered as two leading candidates. Here, we outline the biological traits of these energy crops for large-scale production in China. We also review recent progress on understanding of plant cell wall composition and wall polymer features of both plant species from large populations that affect both biomass enzymatic digestibility and ethanol conversion rates under various pretreatment conditions. We fnally propose genetic approaches to enhance biomass production, enzymatic digestibility and sugar-ethanol conversion effciency of the energy crops.

Keywords: sweet sorghum, , bioenergy crops, biofuels, plant cell wall, biomass saccharifcation, ethanol conversion

1. Introduction

Bioenergy is regarded as a sustainable alternative to fossil energy supply (Chen and Peng 2013; Cotton . 2013). As the second largest energy consumer globally, China has launched several non-fossil energy developing plans, including the 11th Five-Year Plan for Energy Development Planning of China (NDRC 2007a), and the Medium- and Long-Term Developmental Plan for Renewable Energy in China (NDRC 2007b).

To reach the goals outlined in these plans, the selection of bioenergy crops is an important priority to meet the need of biomass production. In general, bioenergy crops can be classifed as starch-producing crops, sugar-producing crops and lignocellulose-rich crops for bioethanol production, as well as oilseed crops for biodiesel (Li . 2010). Starch or sugar-based bioethanol and edible-oil-derived biodiesel may, however, impose challenges for food security if produced on a large scale in China. Nevertheless, conversion of lignocellulosic residues from food crops is a potential alternative (Xie and Peng 2011). Despite those approximately 0.7–0.9 billion tons of crop residues are produced each year, almost half of the residues are burnt to ash or directly discarded around the feld (Chen . 2009). In addition, approximately 0.1 billion ha of marginal lands not suitable for food crops can be applied to grow energy

Received 16 April, 2015 Accepted 26 August, 2015
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doi: 10.1016/S2095-3119(15)61181-9

crops to meet the large demand of biomass feedstock in China (Yan . 2008). We argue that sweet sorghum and should be considered as major candidates of non-food energy crops for marginal lands.

2. Biological characteristics of sweet sorghum and *Miscanthus*

With the advances of bioefnery technologies of converting biomass into biofuels, efforts have been made to grow dedicated biomass crops in China. Sweet sorghum and , which are respectively originated in Africa and East Asia, are the candidate crops with extremely high biomass yields. Moreover, as these two species are evolutionary related, research advances in each of the crops will expedite improvement in the other crops (Van der Weijde . 2013).

Sweet sorghum grows rapidly (a life-cycle is around 120–150 days), and has high biomass yield (6.0–7.5 t dry matter (DM) ha^{-1} yr^{-1}). Sweet sorghum is, furthermore, highly water-usage effcient, and needs typically only one third of water compared to sugarcane and half of that of corn (Li J 2013). It is also drought, salt and cold tolerant as compared to conventional bioenergy crops (e.g., sugarcane and corn). As sweet sorghum can adapt to various environments with low fertilizer requirements, it is extensively grown globally, and is particularly well suited for agriculture in the north of China (Li and Chan-Halbrendt 2009; Xie and Su 2012). Li . (2014a) and Wu . (2015) examined over 200 sweet sorghum germplasm accessions stored in the National Plant Germplasm System of China. These accessions were collected from across the world and displayed clear differences in agronomic trait, such as plant height, stem diameter, pitch numbers, lodging resistance, soluble sugar levels and seed yield. From such germplasm collections, it may therefore be possible to fnd dedicated sweet sorghum accessions that are rich in soluble sugars and that have high digestible lignocellulosic bagasse suitable for bioenergy purposes (Byrt . 2011; Zegada-Lizarazu and Monti 2012; Li . 2014a).

is also a typical C_4 plant that grows rapidly with low fertilizer requirement and high tolerance/resistance to drought, salt and cold conditions. It has wide geographic distributions and high biomass yields ranged from 37.5 to 60.8 t DM ha^{-1} yr^{-1}. For instance, the natural distribution of in China is 100.45–127.55°E, 18.34–43.70°N, alititude –12–1 900 m across 23 provinces (Table 1). So far, eleven species of have been identifed (Jakob . 2009), and over 1 400 natural accessions, including four different species (*sacchariforus*, , , and *foridulua*) have been collected in China (Xie and Peng 2011).

Regardless of the relatively low soluble sugars in the stalks compared with sweet sorghum, is considered as a leading lignocellulosic bioenergy crop in China, and across the world (Lewandowski . 2003; Angelini . 2009; Xie and Peng 2011). While is mainly exploited for lignocellulosic biomass, sweet sorghum, maize, and sugarcane are dual-purpose crops for foods and biofuels (Table 1).

3. Bioethanol production from lignocellulosic residues of sweet sorghum and *Miscanthus*

Various technologies have been applied to enhance biomass enzymatic saccharifcation and ethanol conversion effciency. Sweet sorghum contains approximately 160–180 g L^{-1} fermentable sugars, including sucrose, glucose and fructose, in the stalk juice (Laopaiboon . 2009), which can be readily converted into ethanol by yeast fermentation (Sipos . 2009; Ratnavathi . 2010). It is also an ideal substrate for fuel gas production, such as hydrogen, by biomass gasifcation (Antonopoulou . 2008). A two-step membrane separation process has been developed to increase sugar concentrations and thus ethanol productivity from the stalk juice (Sasaki . 2014). The remaining bagasse of sweet sorghum is lignocellulose-rich which can also be processed to ethanol. To enhance the enzymatic digestibility of sweet sorghum bagasse, various pretreatment methods have been examined. Dilute NaOH solution autoclaving and H_2O_2 immersing pretreatment signifcantly increased cellulose hydrolysis yield, total sugar yield and ethanol concentration by approximately 6-, 10- and 20-folds, respectively, compared with the control (Cao . 2012). Integrating hydrothermal pretreatment and alkaline post-treatment signifcantly increased the saccharifcation ratio of sweet sorghum bagasse (Sun . 2015). Steam-pretreatment also resulted in effcient enzymatic hydrolysis of bagasse and conversion of 85 to 90% of the bagasse into ethanol (Sipos . 2009). Integration of solid-state fermentation technology and alkaline pretreatment has been shown to be a cost-effective process for the production of the ethanol from the sweet sorghum bagasse (Li J . 2013). In addition, sweet sorghum stalk has been examined as the feedstock for methane (Matsakas . 2014) and hydrogen production (Antonopoulou . 2008). It has also been used for heat production (Sipos . 2009). Sweet sorghum produces grains at a yield of about 2.2–4.5 t DM ha^{-1} yr^{-1}, which can be used as food as well as the feedstock for bioethanol and pigment production (Gao . 2010).

Unlike sweet sorghum, is a dedicated

lignocellulosic crop. Field trials in Europe during the last 15 years with the sterile, triploid hybrid (Clifton-brown . 2004; Heaton . 2004) have produced annual harvestable yields that range from 10 to 40 t DM ha^{-1} yr^{-1}; more than double that of switchgrass. One ton of could produce up to 80 gallons of cellulosic ethanol (Lewandowskiet . 2000). Compared with maize ( ), ( ) is almost 60% more productive (Dohleman and Long 2009). An almost complete digestion (95%) was achieved by employing a two-stage method (alkaline peroxide and electrolyzed water). This was a better yield than the use of 1% H_2SO_4 pretreatment (200°C, 8 min) (Wang . 2010). Various chemical and physical pretreatments have also been applied to enhance biomass enzymatic digestibility and ethanol production from (Zhang . 2013; Li . 2014b). However, harsher pretreatment conditions are required in than that of sweet sorghum bagasse, probably due to its distinct biomass recalcitrance.

4. Lignocellulosic features affecting bio-

that the DP of cellulose also affects the biomass saccharification negatively in sweet sorghum, and other plants (Yang . 2011; Wu . 2013; Zhang . 2013; Li . 2014b). This is likely due to the fact that reduced cellulose DP increases cellulose chain-reducing ends and therefore reduces cellulose crystallinity (Zhang and Lynd 2004; Pan . 2008). In addition, the mole number (MN) of cellulose is an important parameter that influences biomass enzymatic digestibility. The cellulose MN can be determined by dividing cellulose content per unit length by mole weight of cellulose (Kokubo . 1991). In , the MN correlates negatively with biomass enzymatic digestion after pretreatments with NaOH and H_2SO_4 (Zhang . 2013).

Hemicelluloses are a class of heterogeneous polysaccharides with various hexose and pentose units. In grasses, xylans are the major hemicelluloses and are commonly substituted with α-L-arabinofuranosyl units on the C2- and/or C3-position (Girio . 2010; Scheller and Ulvskov 2010). Hemicelluloses are generally believed to provide cross-linking interactions with cellulose and lignin, which strengthens the cell wall and possibly function as molecular spacers for cellulose microfibrils. Using large numbers of accessions with diverse cell wall compositions, hemicelluloses were found to be a predominant factor that positively determines biomass enzymatic digestibility after pretreatments with NaOH and H_2SO_4 by reducing cellulose crystallinity (Xu . 2012). Furthermore, a higher degree of arabinose substitution of xylan (reverse Xyl/Ara) positively influenced biomass digestibility in (Li F . 2013). Here, hemicelluloses with high arabinose levels correlated negatively with cellulose crystallinity and enhanced both plant lodging resistance and biomass enzymatic digestibility in rice (Li . 2015). In sweet sorghum and wheat, a high arabinose substitution degree of non-KOH-extractable hemicelluloses can also enhance biomass enzymatic digestibility by reducing cellulose crystallinity (Wu . 2013; Li . 2014a).

Lignin is a stable and complex polymer consisting of three major phenylpropane units: -hydroxyphenyl (H), guaiacyl (G), and syringyl (S) (Sun . 2013). As lignin is associated with other wall polymers ester- and ether-linked bonds, it acts as barriers that hinder enzyme penetration to access cellulose surfaces (Achyuthan . 2010). Due to its structural diversity and heterogeneity, lignin has multiple roles in biomass enzymatic digestions. For example, increased S/G ratios negatively affect digestibility of biomass, whereas increased H/G ratios positively affects saccharification of rice and wheat biomass (Xu . 2012; Wu . 2013; Jia . 2014; Li . 2014a). Although lignin did not appear to influence cellulose crystallinity in sweet sorghum, high levels of lignin G-monomers had a negative impact on biomass digestion, and the release of G-monomers from the biomass significantly inhibited yeast fermentation (Li . 2014b). In , the minor wall-networks between monolignols and inter-linked-phenolics predominantly affects biomass digestibility, and mild alkali-pretreatment effectively extracts guaiacyl-rich lignin for high lignocellulose digestibility coupled with largely diminishing yeast fermentation inhibitors (Li . 2014b). In addition, lignin extraction enhances biomass enzymatic saccharification in hemicelluloses-rich species under various alkali and acid pretreatments (Si . 2015).

In conclusion, reduced CrI/DP and increased arabinose substitution degree of xylans positively influence biomass enzymatic saccharification under various pretreatments in both sweet sorghum and , whereas high levels of G-monomers and low S/G ratios of lignin negatively affect biomass digestibility, respectively (Table 2). This suggests that optimizing certain wall characteristics will make sweet sorghum and more suitable as the feedstock for liquid biofuel production.

5. Biotechnology for sweet sorghum and *Miscanthus* bioenergy breeding

As large populations of natural germplasm accessions of

Table 2 Effects of cell wall composition and polymer features on biomass saccharification in sweet sorghum and

Plant species	Cell wall polymers	Cell wall composition (% dry matter)	Polymer features[1)]	Impacts on biomass saccharification	References
Sweet sorghum	Cellulose	27–37	CrI, DP	Negative	Yang . (2011); Li . (2014a); Wang . (2014)
	Hemicellulose	29–33	Reverse Xyl/Ara	Positive	Li . (2014a); Wang . (2014)
	Ligin	17–20	G, S/G	Negative	Li . (2014a); Wang . (2014)
	Cellulose	28–49	CrI, DP, MN	Negative	Zhang . (2013); Van der Weijde . (2013); Wang . (2014)
	Hemicellulose	24–32	Reverse Xyl/Ara	Positive	Xu . (2012); Li F . (2013); Wang . (2014)
	Ligin	15–28	S/G	Negative	Li . (2014b); Li Z . (2014); Wang . (2014)

1) CrI, cellulose crystallinity index; DP, degree of polymerization of crystalline cellulose; Reverse Xyl/Ara, degree of arabinose substitution of xylan; G, guaiacyl; S/G, syringyl/guaiacyl ratio; MN, the mole number of cellulose.

sweet sorghum and have exhibited a diverse cell wall composition and biomass sacchari f cation, it may be appropriate to screen for high biomass digestibility for biofuel production. However, traditional screening approaches are labor-intensive, time-consuming and expensive as it includes chemical analyses of plant cell wall compositions and estimates of total sugar yields released enzymatic hydrolysis (Roberts . 2011; Li . 2014b). Recently, near infrared spectroscopy has been used for high-throughput screening of sweet sorghum and accessions (Huang . 2012; Wu . 2015). Using 199 accessions, seven optimal models were iden f ed with high determination coef f cient for biomass enzymatic digestibility upon various physical (heat) and chemical (1% NaOH, 1% H_2SO_4) pretreatments (Huang . 2012). In addition, a total of 123 sweet sorghum accessions and 50 mutants were examined for stalk soluble sugars, bagasse enzymatic sacchari f cation and wall polymer features. From these measurements, calibration equations were generated that can effectively determine the relationships between stalk soluble sugars, bagasse enzymatic sacchari f cation and cell wall polymers (Wu . 2015).

is a natural hybrid, and has more than 20 excellent biological characteristics. Over the past years, various new technologies of biomass pretreatments have been applied in sweet sorghum and to enhance biomass enzymatic digestibility and to reduce ethanol conversion cost by yeast fermentation. Genetic modifcations that affect the plant cell wall have been proposed as holding great promise to overcome biomass recalcitrance by reducing cellulose crystallinity, increasing arabinose substitution degree of xylans, or altering the relative proportions of the three monolignols in lignin in sweet sorghum and . Furthermore, screening of large populations of natural germplasm accessions and cell wall mutants is an alternative approach to identify new lines with improved saccharifcation rates. Molecular breeding will be a powerful approach to develop new varieties for bioenergy production in sweet sorghum and .

Acknowledgements

This work was supported by grants from the Fundamental Research Funds for the Central Universities Project , China (2013QC042), the Fundamental Research Funds for the 111 Project of Ministry of Education of China (B08032), and the Starting Foundation for Changjiang Scholars Program of Ministry of Education of China (52204-14004).

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(Managing editor SUN Lu-juan)