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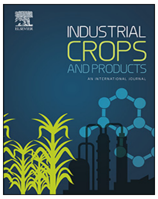

Physico-chemical characterization of pedigreed sorghum mutant stalks for biofuel production

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ABSTRACT

The successful commercialization of cellulosic biofuels depends on a stable and sustainable supply of high quality biomass at a reasonable cost. The physical property and chemical composition of biomass are the most important indicators of a biomass source's potential for biofuel application. This study characterized the physical and chemical properties of 148 pedigreed sorghum (*Sorghum bicolor* L.) mutant stalks for biofuel production in order to accelerate the discovery of genes or gene mutations that underlie significant beneficial traits in sorghum. Large variations of chemical compositions were observed and indicated that breeding progress was achievable for sorghum development with desirable biofuel properties. The extractives are negatively correlated with the cellulose, hemicellulose, and lignin contents with an r of -0.85, -0.87, and -0.94, respectively. However, no relationships between ash and extractives contents were found in this study. Among the tested 148 sorghum mutant stalks, sorghum mutant ARS14 was identified with high sugar contents, high heating values, but less ash contents, which was suitable for the biorefinery industries with both biochemical and thermochemical conversions.

1. Introduction

As the global population expands, the energy consumption increases, petroleum reserve decreases, and the demands to produce more renewable fuels increase rapidly. Among a number of potential alternative fuels, bioethanol is considered as the most wildly utilized transportation fuel. Ethanol can function as an octane booster to resist engine knocking and contribute to national energy security. Bioethanol is a renewable alternative fuel derived from various sustainable feedstocks such as sugar-based crops such as sugarcane (*Sacharum officinale* L.), sweet sorghum (*Sorghum bicolor* L.), and sugarbeet (*Beta vulgaris* L.); starch-based crops such as wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), cassava (*Manihot esculenta* L.), and grain sorghum; cellulosic biomass such as corn stover and wheat straw; herbaceous biomass such as switchgrass (*Panicum virgatum* L.), big bluestem (*Andropogon gerardi* L.), sorghum stalk, and miscanthus; and woody biomass such as hybrid poplar and willow (Goshadrou et al., 2011; Limayem and Ricke, 2012; Parawira and Tekere, 2011; Wu et al., 2010).

Sorghum is a C4 photosynthetic species, which is highly productive and well adapted to arid growing conditions. Some unique characteristics of sorghum have designated it as a potential bioenergy crop, including high yields and carbohydrate contents, high water-use efficiency and strong drought tolerance, well-established production and management system, and potential for genetic improvement. While sorghum is well known as a cereal grain, it's also grown as energy crop lately. Similar as other crop residues, sorghum biomass is mainly composed of cellulose, hemicellulose, and lignin, in which both cellulose and hemicellulose are polysaccharides, and thus is a potential source of fermentable sugars (Balat and Balat, 2009). Cellulose is a polymer of glucose with β-1,4 linkage and is found in both the crystalline and non-crystalline structures (Ioelovich, 2008). Properties of cellulose are highly linked to its degree of polymerization (DP), which represents the number of glucose monomers that make up one polymer molecule. Cellulose is insoluble both in water and dilute acid solutions at room temperature, but in alkaline solutions, swelling of cellulose occurs and cellulose with low molecular weight ($DP < 200$) is dissolved (Badger, 2002; Ioelovich, 2008). Hemicelluloses isolated from agricultural residues could be potential sources of xylans, which are the linear β-1,4-linked xylopyranosyl main chains. Xylans are heteropolysaccharides with homopolymeric backbone chains of β-1,4-linked D-xylopyranose units, which contain xylose, arabinose, glucuronic acid or its 4-*O*-ether, and acetic, ferulic, and *p*-coumaric acids (Saha, 2003;

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Scheller and Ulvskov, 2010). The distribution pattern of side chains in heteroxylans, which reflects the structure of the polymer chains, has major influences on their solubility, interactions with other cell wall polymers, digestibility of enzymes, rheological properties, and other functional properties (Gáspár et al., 2007; Saha, 2003; Scheller and Ulvskov, 2010). Lignin is a complex polymer of phenylpropanoid units and its presence in the cell wall provides carbohydrate-lignin seal protection, thus inhibiting the enzymatic hydrolysis of cellulose and hemicellulose (Azadi et al., 2013; Li et al., 2014). Cellulose content of agricultural residues was usually reported in the range of 25–35%, whereas hemicellulose constitutes 20–30% in biomass. Structural carbohydrates are an essential source of biomass for bioenergy production, which is the only type that has sufficient feedstocks to produce required bioenergy in the future. Cellulose-based biomass sorghum is necessary to be pretreated in order to open its complex structure for the enzymatic hydrolysis and subsequent fermentation processes.

Biomass is of great importance to provide renewable energy to produce heat and power via combustion (Bridgwater, 2003; Demirbas, 2004). High heating value (HHV), also called gross calorific value, is one of the most important properties of biomass fuels and represents the enthalpy of complete combustion of a fuel, including the condensation enthalpy of formed water (Friedl et al., 2005; Sheng and Azevedo, 2005). Calorific value of biomass is the energy of chemically bound biomass and converted into heat energy after combustion. Many models have been developed to relate the heating value with the elemental composition including C, H, N, S, Cl, and O (Friedl et al., 2005), chemical composition including extractives, cellulose, hemicellulose, lignin, and ash (Telmo and Lousada, 2011), and data from proximate analysis including volatile matter, ash, and fixed carbon (Erol et al., 2010; Nhuchhen and Salam, 2012).

Although sorghum has been emerged as a promising bioenergy feedstock and model for other C4 crops with many complex genomes (de Siqueira Ferreira et al., 2013; Rooney et al., 2007), most genes regulating bioenergy traits are unknown. Sorghum mutant library, especially those that have been sequenced at the whole genome level, provides a useful resource to identify those gene important for biomass production and quality (Jiao et al., 2016; Xin et al., 2008, 2009). Identification of those genes from sorghum may also help improve bioenergy production from other C4 bioenergy crops such as miscanthus and switchgrass.

The objective of this research was to develop a comprehensive understanding of utilization of pedigreed sorghum mutant stalks for biofuel production. Thus, 148 sorghum mutant stalks without panicle and leaves were selected from a sequenced pedigreed sorghum mutant library consisting of 256 individually mutagenized M_4 seed pools for chemical compositional analysis, including water- and ethanol-soluble extractives, structural carbohydrates such as cellulose and hemicellulose, acid-soluble and acid-insoluble lignin, and ash (Jiao et al., 2016). Energy content of each sorghum sample was also measured and reported as HHV.

2. Materials and methods

2.1. Materials

One hundred and forty eight pedigreed sorghum mutant stalks were provided by the Plant Stress and Germplasm Development Unit of USDA-Agricultural Research Services (Lubbock, TX). The sorghum lines were field grown at the USDA-ARS Plant Stress and Germplasm Development Research Unit, Lubbock, Texas (latitude 33° 35′N, longitude 101° 53′W, and altitude 958 m) in 2016 growing season. The soil type is an Amarillo fine sandy loam (fine-loamy, mixed, superactive thermic Aridic Paleustalfs). Before planting, a mixture of bulk ammonium sulfate and mono ammonium phosphate was applied to the field, calculated to achieve a level of 65 kg N/ha and 27 kg P/ha. A randomized complete block design with four replicates was used. The plot size was four rows of 4.67 m long with 1.02 m row spacing. Sorghum seeds were planted at 80 per row at a depth of 3 cm using a John Deere MaxEmerge Planter. The irrigated plots received 5 mm of water per day from underground drip lines located on 1.02 m centers as needed. The drought stressed plots received no irrigation after planting. The plants were harvested at physiological maturity. After removing panicles and leaves, the stalks were dried in a forced-air oven at 60 °C for one week and then stored in the room temperature.

After grinding the sorghum stalks into < 1 mm particle size using a cutting mill (SM 2000, Retsch Inc., Newton, PA), the stalk samples were sealed in a plastic bag and stored at room temperature. The moisture content was determined using the conventional air oven drying method as described in the National Renewable Energy Laboratory (NREL) laboratory analytical procedure (LAP) “Determination of Total Solids in Biomass” (Sluiter et al., 2005). All chemicals used for this research were purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Compositional analysis

2.2.1. Extractives

Two-step solvent extraction using water and subsequent ethanol was applied to determine the extractives content of sorghum mutant stalks. Five grams of sorghum samples was weighed into a Soxhlet thimble and extracted with distilled water for 8 h according to the NREL LAP “Determination of Extractives in Biomass” (Sluiter et al., 2008a). Heating mantles were adjusted to 5 siphon cycles per hour. When the reflux time was complete, the heating mantles were turned off to allow glassware to cool down. Residual water from the Soxhlet tube was removed and ethanol solvent was added, then the ethanol extraction was continued for 16 h. The percentage of extractives was calculated as the dry weight of samples per sample weight as received. Extractives consist of water- and ethanol-soluble materials. Water-soluble extractives contain inorganic materials, non-structural sugars, such as sucrose, fructose, and glucose, and nitrogenous materials. Inorganic materials of water-soluble extractives are from biomass itself or extraneous soil and fertilizer. Ethanol-soluble extractives include chlorophyll, waxes, and other minor components.

2.2.2. Structural carbohydrates

The chemical composition of sorghum mutant stalks was determined according to the NREL LAP (Sluiter et al., 2008b). In the procedure, extraction-free sorghum samples (300.0 ± 10.0 mg) were weighed into each pressure tube and treated by two-step acid hydrolysis with 3.00 ± 0.01 mL sulfuric acid (72%, w/w) at 30 °C for 60 min and hydrolyzed by dilute acid (4%) at 121 °C for another 60 min. Dilute acid (4%) was achieved by adding 84.00 ± 0.04 mL distilled water using an burette. After acid hydrolysis, carbohydrates including cellulose and hemicellulose were converted to monosaccharides, which were measured by high-performance liquid chromatography (HPLC) equipped with an RCM monosaccharide column (300 × 7.8 mm) (Phenomenex, Torrance, CA) and a refractive index detector. The mobile phase was 0.6 mL/min of HPLC grade water, and the column temperature was set as 80 °C.

2.2.3. Lignin and ash

Vacuum filtration was applied to the hydrolyzed solution for collection of the acid-insoluble solids, which include acid-insoluble lignin and ash, whereas acid-soluble lignin in the hydrolysis liquor was determined using a UV/visible spectrophotometer at the 320 nm wavelength with the background of distilled water. Lignin consists of acid-insoluble and acid-soluble lignin. Acid-insoluble lignin was weighed from the remaining solids after the oven heating overnight at 105 °C (the weight of acid-insoluble lignin and ash) and then at 575 °C using a muffle furnace for at least 6 h to measure the ash content.