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Sunflower as a biofuels crop: An analysis of lignocellulosic chemical properties

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ARTICLE INFO

Article history:
Received 3 February 2013
Received in revised form
19 June 2013
Accepted 22 June 2013
Available online 12 August 2013

Keywords:
Biomass
Lignocellulosic biofuel
Lignin
S/G-lignin
Sugar content
Pyrolysis Molecular Beam Mass Spectrometry

ABSTRACT

Four accessions of cultivated sunflower (*Helianthus annuus*) and silverleaf sunflower (*Helianthus argophyllus*), were each grown in three locations (Georgia, British Columbia, and Iowa) at different planting densities and phenotyped for biomass-related traits and wood biochemistry. In most environments, *H. argophyllus* produced significantly more biomass than *H. annuus*. Cell wall chemistry for a subset of plants grown in Georgia and Iowa was assessed using analytical wet chemistry methods to measure lignin and sugar content/composition. The analysis of lignin and the S/G-lignin ratios for a larger number of samples ($n > 250$) was also assessed by high-throughput pyrolysis Molecular Beam Mass Spectrometry. Average pyMBMS estimated lignin content (i.e., dry weight fraction) for 60 °C dried basal stem samples of *H. annuus* and *H. argophyllus* was 29.6% (range, 24.0%–34.6%) and 28.6% (range, 24.6%–33.3%), respectively when averaged across all environments. The average S/G lignin mass ratio was 1.5 (range, 1.0–2.0) for *H. annuus* and 1.7 (range, 1.0–2.4) in *H. argophyllus*. Stem samples from these two species only differed statistically for a few cell wall chemistry traits; however, accession level differences within each species were apparent. Cell wall chemistry in both species was significantly affected by both location and planting density, thus demonstrating the need to select for these traits in the environment for which the crop will be produced. Overall, these results show that cultivated sunflower and silverleaf sunflower both possess the necessary phenotypic diversity to facilitate the development of a hybrid sunflower with improved lignocellulosic biofuels traits, namely increased biomass, decreased lignin, and increased glucan.

Abbreviations: ANN, cultivated sunflower; ARG, silverleaf sunflower; GA, Georgia; IA, Iowa; BC, British Columbia; pyMBMS, pyrolysis Molecular Beam Mass Spectrometry; S-lignin, syringyl lignin; G-lignin, guaiacyl lignin.

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http://dx.doi.org/10.1016/j.biombioe.2013.06.009

1. Introduction

The shift from starch and simple sugar based production of ethanol to lignocellulosic fuels is essential to protecting the world's food and feed supply while still enabling the bulk production of biofuels. Corn stover, grain straws, forestry waste, and purpose-grown lignocellulosic feedstocks (e.g., switchgrass) are vital to maintaining those supply chains; however, there is potential for other crops to play a significant role. Cultivated sunflower (*Helianthus annuus* L.) is a globally important oilseed crop with 24 million hectares harvested in 2010 [1]. In the US in the same year 750,000 ha were harvested with primary production areas in North Dakota, South Dakota, Kansas, and Colorado [2]. The annual yield of residual sunflower biomass after the seed is harvested is estimated to be 3–7 t ha^{-1} [3], which is roughly comparable to corn stover and wheat straw (i.e., 8.4 and 6.0 t ha^{-1}, respectively) [4]. Based on these estimates the annual amount of available sunflower biomass in the US is approximately 3.75 Mt.

Cultivated sunflower is highly adaptable and can be productive on lands with limited inputs [3,5,6]. Silverleaf sunflower (*Helianthus argophyllus* Torr. & Gray) is a closely related [7], drought resistant wild species [8] that produces larger, more solid stems and grows up to 4.5 m tall at higher latitudes in the US and Canada. *H. argophyllus* is interfertile with *H. annuus* [9,10] and could potentially supply the genetic diversity necessary for developing high biomass, woody stemmed cultivars of cultivated sunflower that could be used as a source of lignocellulosic biomass. To maximize economic feasibility, the resultant biomass should possess favorable characteristics such as lower lignin content [11,12] for lower recalcitrance to pretreatment, higher sugar content (especially glucose) for optimal sugar yield [13], and a high ratio of syringyl-lignin subunits to guaiacyl-lignin subunits (S/G-lignin ratio) as this variable has been associated with decreased recalcitrance to pretreatment [14]. The work described herein is aimed at characterizing the biomass properties of multiple accessions of *H. annuus* and *H. argophyllus* with the goal of identifying accessions with desirable chemical characteristics.

Despite its importance as a global oilseed crop, little is known about the chemical composition of sunflower biomass. While a recent study [15] described the composition of sunflower stalks, this work only focused on a single accession of sunflower and did not provide an assessment of genotype by environment effects (G × E) or the variation across the sunflower gene pool, much less in related species. This report is the first to our knowledge to assess variation in basal stem composition in both *H. annuus* and *H. argophyllus* grown in multiple locations at different planting densities.

In this study, we investigated differences in the cell wall chemistries and growth patterns of eight *Helianthus* accessions (i.e., four *H. annuus*, four *H. argophyllus*) as a first step in understanding the potential of sunflower as a source of lignocellulosic biomass for biofuels production. We conducted an in-depth analytical characterization (e.g., lignin and sugar content and composition) of a subset of plants in addition to a more comprehensive study of >250 plants using high-throughput pyrolysis Molecular Beam Mass Spectroscopy (pyMBMS) to assess lignin content and composition. The applicability of using high-throughput pyMBMS on these samples is demonstrated by the high correlation ($R^2 = 0.87$) found in this study between traditional Klason lignin results and the pyMBMS results. The findings from this study provide insight into the genetic and non-genetic factors affecting lignocellulose accumulation, plant cell wall formation, biomass yield, and other cellulosic biomass traits in sunflower.

2. Materials and methods

2.1. Plant material and planting design

In 2009, four accessions of *H. argophyllus* (derived from wild collected, open-pollinated populations) and four accessions of *H. annuus* (two elite inbred lines, one Native American landrace, and one wild accession) (Supplemental Table S1) were planted in GA (Plant Sciences Farm, Watkinsville, 33°52′20″N and 83°32′08″W), IA (North Central Regional Plant Introduction Station, Ames, 42°00′43″N and 93°39′32″W) and Vancouver, Canada (University of British Columbia Farm, 49°15′03″N and 123°14′20″W). Sites were chosen to represent a broad range of environments where sunflower could be produced. Accessions were selected to represent variation in flowering phenology, growth habit, and geographical origin within both species [16]. The wild accessions were pre-germinated following standard protocols to overcome seed dormancy issues and to maximize seedling establishment. These seedlings were subsequently transplanted into greenhouse trays and moved to the greenhouse for 2–3 weeks and then transplanted into the field. In IA and BC, seeds of the *H. annuus* inbred lines and the Native American landrace were germinated in the greenhouse in greenhouse trays and transplanted to the field after emergence, while in GA these accessions were sown by hand directly into the field.

In GA and IA, row plots were established with 3 different planting densities with 0.3, 0.9, and 1.5 m between plants within a row plot and 3.0 m between plots. Due to space constraints at the BC location, only the middle planting density of 0.9 m between plants was planted. Twenty plants of a single accession were planted in each row plot. The row plots were randomized within a block with two blocks planted per location. The row plots for the 0.3, 0.9, and 1.5 m planting densities were 6.1, 18.3, and 30.5 m in length, respectively. Plants were phenotyped and harvested for chemical analysis only if they appeared to be growing normally and at the prescribed planting densities; end row plants were not included (Supplemental Table S2). Roughly 75% of the plants at the GA location were severely damaged by insect feeding, so the sample sizes from this location were small (Supplemental Table S2) and were only analyzed using analytical wet chemistry techniques.

Plant height was measured at flowering (R5.1) [17]. The number of days to flowering was recorded from the field planting dates used in each location. Basal stem sections (i.e., 0.3 m in length) were manually harvested at maturity (R9)