



## Contributions of environment and genotype to variation in shrub willow biomass composition



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### ABSTRACT

Yield improvement of woody bioenergy crops has been the major focus of breeding programs, but biomass quality is also important for conversion to biofuels. Using high-resolution thermogravimetric analysis, the composition of biomass samples from two shrub willow (*Salix* spp.) yield trial networks representing two distinct datasets were examined. Dataset 1 consisted of 12 yield trials containing 10 genotypes that mainly represented early cultivars from the US breeding program. Dataset 2 consisted of five trial locations containing 19 genotypes from later breeding efforts. Variation in ash and hemicellulose content were largely controlled by genetic factors, while cellulose and lignin content were heavily influenced by environmental effects. Mean biomass composition traits for dataset 1 were 2.1% ash, 42.4% cellulose, 18.7% hemicellulose and 25.5% lignin. For dataset 2 mean traits were 1.9% ash, 43.2% cellulose, 17.7% hemicellulose and 25.7% lignin. Yield was negatively correlated with lignin content and positively correlated with cellulose content at the level of environment. Elite triploid hybrid cultivars have the potential to produce more cellulose per unit area because of higher yields and/or greater cellulose content. These findings suggest that selection of genotypes for improved yield, as well as selection of growing environment, can improve feedstock quality for biofuel production.

### 1. Introduction

Concerns over rising atmospheric carbon dioxide levels and the detrimental impacts associated with anthropogenic climatic change, coupled with interests in energy independence have led to national efforts to explore alternative sources of transportation fuels as viable replacements for petroleum-based fuels (Mussatto et al., 2010). Currently, traditional agricultural crops represent the major source of liquid biofuels, so called first-generation biofuels. In the US, current biofuel production mandates are targeted at approximately 68 GL per year, with the vast majority of total volume (80%) derived from maize grain (Environmental Protection Agency, 2015). Lignocellulosic material, in the form of plant stems and leaves, represents an abundant source of reduced carbon available for the production of second-generation biofuels. First-generation biofuels are widely criticized for competing with food production (Graham-Rowe, 2011) and result in lower greenhouse gas emission reductions compared with second-

generation lignocellulosic feedstocks (Cherubini and Strömman, 2011). Due to these concerns, first-generation biofuel production in the US is capped at the present-day production level of 55 GL, and statutes mandate that second-generation biofuel production will match those levels by 2022. According to the most recent US government assessments of national biomass production potential, dedicated energy crops can provide the majority of biomass resources needed for bioenergy production in the coming decades (U.S. Department of Energy, 2016). However, this will require enormous investments in the expansion of operations across the supply chain.

Perennial woody bioenergy crops, such as shrub willow (*Salix* spp.), have key aspects relating to sustainability, including favorable greenhouse gas balances (Djomo et al., 2015), high rates of soil carbon storage (Cunniff et al., 2015; Pacaldo et al., 2014), low nutrient demand (Aronsson et al., 2014) and water quality benefits (Kuzovkina and Quigley, 2005; Styles et al., 2016). Recent life cycle analyses have concluded that yield is an important determining factor relating to

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greenhouse gas balances, and that fertilization will likely have detrimental effects (Caputo et al., 2014; Krzyżaniak et al., 2016). These benefits together with the pressing needs to meet federal mandates and to control the concentration of carbon dioxide in the atmosphere will necessitate the expansion of robust, commercial-scale conversion technologies, which will demand steady supplies of feedstocks with uniform quality. Therefore, characterization of feedstock quality and an understanding of the sources of variability are of utmost importance.

Plant cell walls represent a vast reservoir of reduced carbon in the form of biopolymers, mostly cellulose, hemicellulose and lignin bound together in a complex network. Cellulose is the most abundant biopolymer on Earth and provides structural rigidity to plant cell walls. It is also a significant source of carbohydrates available for enzymatic hydrolysis and fermentation into liquid fuels, and represents the majority of substrates intended for second-generation biofuel production (Somerville et al., 2010). Hemicellulose is a heterogeneous biopolymer that adds strength to cells walls by linking cellulose microfibrils. Its composition differs greatly by plant species, but is mainly comprised of five-carbon sugar monomers and can therefore be a source of fermentable substrate using specialized or engineered microorganisms following chemical hydrolysis. Lignin has many important physiological roles in plants, including providing a hydrophobic surface in vascular tissues for water transport, and structural stability and resistance to disease and pest attack. However, it also presents a significant impediment to enzymatic cell wall depolymerization in liquid fuel production. Lignin has a higher energy density compared with cellulose and therefore is viewed as a desirable component for feedstocks used for thermal conversion. In contrast, ash, or mineral components of biomass, can be problematic for thermal and thermochemical conversion technologies, creating corrosion, slagging or fouling deposits. In biochemical conversion processes, ash can decrease pretreatment efficacy.

Woody biomass crops like poplar (*Populus* spp.) and willow have greater lignin and lower cellulose, hemicellulose and ash contents than perennial grasses (Karp and Shield, 2008) and have typically been considered better feedstocks for thermal conversion. However, recent studies have demonstrated that genetic variability in biomass composition can affect potential biofuel production among species of poplar (Studer et al., 2011) and willow (Brereton et al., 2010; Serapiglia et al., 2013b), encouraging interest in exploring the genetic resources of these woody crops available for liquid fuel production. There is also great interest in the potential for genetic modification of cell wall composition in woody crops to improve conversion efficiency, however, in willow, unlike its close relative poplar, implementation of genetic modification for trait modification has not yet been reliably demonstrated (Stanton et al., 2014). Therefore, selection through traditional breeding techniques remains the most tractable mode for biomass quality improvement. Trait mapping with the aid of genomic resources for willow and poplar have recently been employed (Berlin et al., 2014; Brereton et al., 2010; Hanley et al., 2011), paving the way for marker-assisted selection.

Breeding efforts in North America have focused on improving yields by capturing heterosis in inter-specific hybrid crosses (Fabio et al., 2017b; Serapiglia et al., 2014), but with extensive genetic resources available, efforts have also been made to characterize variation in biomass composition among genotypes. Tharakan et al. (2003, 2005) found significant differences in wood density, bark concentration and ash content among a number of willow genotypes representing multiple species, many of which comprised the foundational material for the US breeding program (Smart et al., 2005). Serapiglia et al. (2013a) examined variability in biomass composition among 18 genotypes from initial breeding work at two locations in NY and found strong genotypic differences in cellulose, hemicellulose, lignin and ash content, as well as significant genotype-by-environment interactions (GEI) for some traits. Some important factors under genetic control that may affect biomass composition include stem diameter, bark proportion (Adler et al., 2005)

and biomass growth rate (Novaes et al., 2010). Besides genetic control, other factors affecting biomass quality include: stand age or rotation length (Adler et al., 2005; Stolarski et al., 2011), soil type (Krzyżaniak et al., 2015; Liu et al., 2016), fertilization rate (Larsen et al., 2016), and mechanical stress resulting in tension wood formation (Brereton et al., 2012). Despite a growing body of literature, most investigations have been conducted at single locations which prevents the evaluation of environmental and GEI effects.

The aim of this research was to quantify and describe the contributions of genotypic, environmental and GEI on shrub willow biomass composition variability, assessed by a high-throughput method. Biomass samples from two complementary, but unique yield trial networks that differ in their relative levels of geographic and genetic diversity, and represent different phases of breeding work in the US (Fabio et al., 2017a). The objectives were to 1) quantify the relative contributions of genetic, environmental and interaction factors in explaining variability in biomass traits; 2) explore patterns of correlation among the measured biomass traits; and 3) determine the degree to which biomass traits are influenced by genetic diversity and breeding.

## 2. Materials and methods

### 2.1. Yield trial networks and field sampling

The first yield trial network represented a combination of natural accessions and intra- and interspecific hybrids selected from early breeding efforts conducted at the State University of New York College of Environmental Science and Forestry, resulting from crosses made in 1998 and 1999. After selections were made based on initial field testing (Smart et al., 2008), yield trials were established in the US and Canada between 2005 and 2011, each containing between 16 and 30 genotypes planted in 78-plant plots. Each plot had three-double rows. Genotypes were arranged in a randomized complete block design with four replicates. The first-year's growth was cut back and subsequently the trials were managed on a three-year rotation. Harvests were conducted on 18–22 plants per plot from the middle double row, either by manually cutting and weighing stems on hanging scales or by mechanical single-pass cut and chip harvesting and weighing chips in a bin fitted with load cells. A subsample of fresh biomass was collected from each plot, either as whole stems or chips depending on harvest method, and used to determine moisture content after oven drying at 65 °C to a constant weight. This was used in order to express yield on a dry-matter basis.

Not all genotypes were present in all locations, and biomass samples for compositional analysis (described below) were not available for all trials in this network, so a reduced set of observations was selected that maximized geographic and genetic variability, while keeping the amount of missing data to a minimum (3.3% missing genotype-environment combinations). The final set of observations included 12 environments from the upper Midwestern and Northeastern US (Table S1) and represented 10 genotypes from five distinct pedigrees, or diversity groups, including two pure species and three inter-specific hybrid diversity groups (Table S2). Hereafter, measurements obtained from this first yield trial network will be referred to as dataset 1.

The second yield trial network consisted of five trials established in 2012 and 2013, each containing 24–27 genotypes representing a combination of commercial cultivars and high-yielding genotypes selected in a trial established in 2008 at Cornell University's New York State Agricultural Experiment Station in Geneva, NY (Serapiglia et al., 2014). The five trials were located in Escanaba, MI, Fredonia, NY (two trials), Geneva, NY and Rock Springs, PA, and were laid out in a randomized complete block design with four replicates (Table S3). In Fredonia, NY, the two trials were established side-by-side, one receiving soil fertility amendments of 1800 kg ha<sup>-1</sup> of lime and 84 kg N, P and K ha<sup>-1</sup> before and after the establishment year, while the other served as an unamended control. Most trials were planted in a double-row

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