SELECTION OF HIGH-YIELDING SHRUB WILLOW GENOTYPES WITH IMPROVED BIOMASS CHARACTERISTICS FOR CONVERSION TO BIOFUELS

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Genetic improvement of shrub willow (*Salix*), a perennial energy crop suited for temperate climates, has led to the development of new cultivars with improved biomass yield, pest and disease resistance, and biomass composition for bioenergy applications (Serapiglia et al., 2009; Serapiglia et al., 2012). Cultivar development, resulting from controlled hybridizations, exploits the high levels of genetic diversity among natural populations of many willow species. Current research efforts continue to focus on improving willow biomass traits important to the renewable fuels industry, including production yield and compositional quality. Species hybridization leading to heterosis has been exploited by many breeders to improve growth and vigor in several tree species, although little is known about the genetic basis for this phenomenon (Li and Wu, 1996; Kopp et al., 2002).

The objective of this study was to evaluate progeny from crosses performed in 2001, 2002, and 2005 and to identify traits associated with heterosis and variation in biomass compositional quality. The genus *Salix* consists of approximately 300 species with a wide range in ploidy levels, including diploids, tetraploids, hexaploids, and even higher levels of ploidy in some cases. Many of the progeny evaluated in this study were triploids, which are known to generally display improved vigor relative to their diploid and tetraploid parents (Zsuffa et al., 1984). The top performing genotypes in this trial, representing advanced pedigrees compared to those in previous trials, were mostly triploid in nature and outperformed current production cultivars.

MATERIALS AND METHODS

A total of 76 genotypes were hand planted in May 2008 as rooted plants started from 10-cm cuttings in double row spacing at a density of 14,350 plants ha^{-1}. Each genotype was planted in 24-plant plots with six plants per row. The trial was a randomized complete block design with a total of three blocks. During the establishment year, a combination of hand-weeding and spot herbicide (glyphosate) application was used for weed management. In December 2008, at the end of the first growing season, the trial was coppiced. Prior to budbreak in the following spring, pre-emergence herbicide (oxyfluorfen) and post-emergence herbicide (clopyralid) were applied to control weeds. In addition, nitrogen fertilizer was applied at a rate of 50 kg ha^{-1}. Growth traits, wood density, and cell wall compositional traits were measured following each growing season (2009, 2010, and 2011). After the third post-coppice season, the above ground biomass was harvested in December 2011. Biomass composition analysis was performed on three-year old biomass using standard wet chemical methods and by a high-resolution thermogravimetric analysis method developed for low-cost phenotyping of shrub willow biomass (Serapiglia et al., 2009). Variation among key traits and their impacts on biomass production were analyzed.

RESULTS AND DISCUSSION

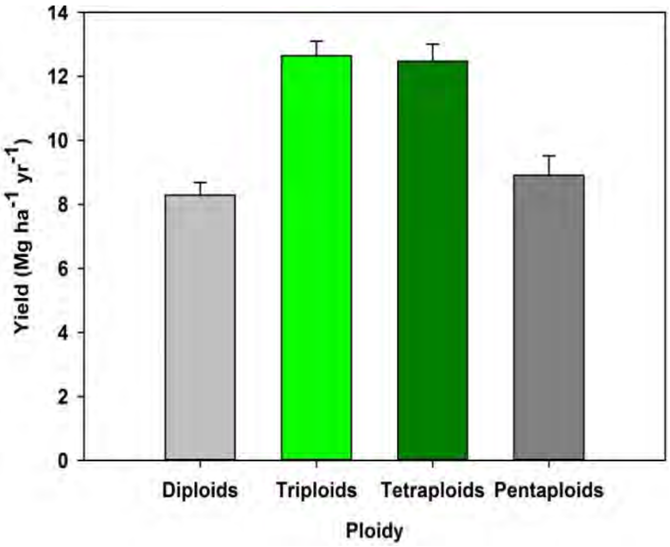

Figure 1 - Harvested biomass at 3-years post-coppice by ploidy of genotype.

Many of the progeny examined in this trial displayed heterosis for yield compared to their parents. Among the 76 genotypes within this study, triploids produced the greatest yields. The top 50th percentile within the trial consisted of mainly triploids and tetraploids, while the diploids and pentaploids were lower yielding genotypes (Fig. 1). The highest yielding genotype in the trial was an interspecific hybrid triploid, (*S. koriyanagi* × *S. purpurea*) × *S. miyabeana,* producing a final yield of 17.4 oven dry Mg ha^{-1} yr^{-1}. Many of the triploid progeny in this trial were from crosses between diploid species, such as *S. purpurea*, and the tetraploid species, *S. miyabeana*. Consequently, most of these triploid genotypes have reduced reproductive fertility (Kopp, 2000), which reduces concerns about potential invasiveness. Current studies are underway to improve our understanding of the molecular basis for heterosis in these highly heterozygous willow triploids. However, due to the high heterozygosity in willow parental genotypes, models for heterosis based on crop systems with highly inbred and homozygous parental lines need to be reconsidered. A clearer understanding of this phenomenon and ability to predict the performance of progeny prior to making a cross will not only improve breeding efforts in willow, but in many other bioenergy crops and tree species.

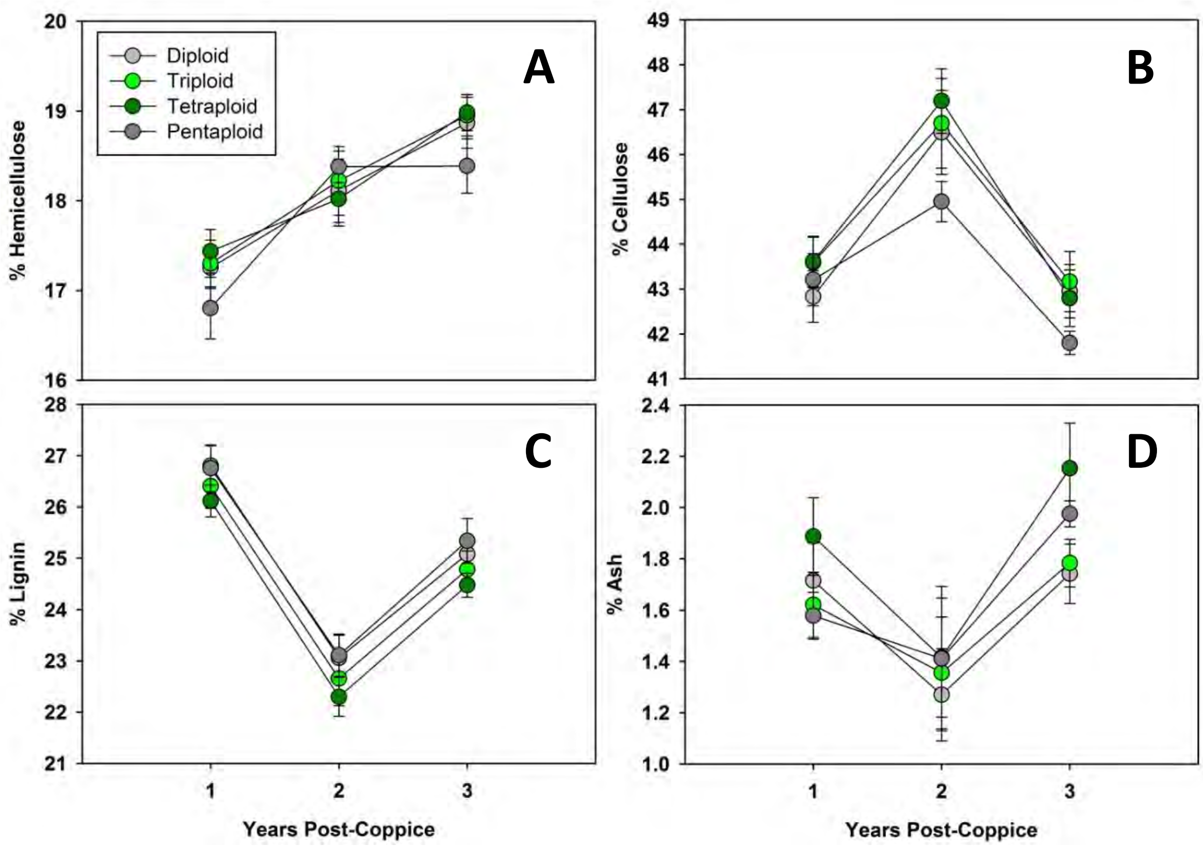

Figure 2 – Changes in biomass composition across all three years by ploidy. A) % Hemicellulose, B) % Cellulose, C) % Lignin, D) % Ash.

The mean yield of the top five performing new genotypes in this trial was 16 oven dry Mg ha^{-1} yr^{-1}, while the mean yield of the top five current commercial cultivars was 14 oven dry Mg ha^{-1} yr^{-1}. This represents an improvement of 12.5 % in yield over current commercial cultivars on this single site. Larger scale yield and evaluation trials with a selection of the top genotypes have been planted in NY, PA, and WV to further test their performance prior to final selection, scale-up, and commercial deployment.

Biomass composition was significantly different across the three-year growth cycle (Fig. 2). Differences in biomass composition among genotypes with different ploidies were also observed. Cellulose content was greater in the second year compared with the first and third years of growth. The opposite pattern was apparent for lignin. There was a significant negative correlation between lignin content and yield over all three years (data not shown). In addition, the triploid and tetraploid genotypes displayed the lowest lignin content regardless of year. Further studies are required to determine if lignin content in the biomass has a negative impact on yield. Through controlled breeding, high-yielding triploid willow cultivars were produced with relatively low lignin content, which has been shown to improve enzymatic saccharification, potentially improving the quality of these cultivars as feedstocks for biochemical conversion to liquid biofuels (Studer et al., 2011).

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