Diallel Analysis of Biomass and Ethanol Yield in Leaves versus Stems of Lowland Switchgrass

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Dedication

This thesis is dedicated to my two pillars, my mother and father, Lisa DeSantis and Ferdinand DeSantis. Their endless support and encouragement have carried me through every bump in the road and inspired me to strive for greatness. I can only wish to be half the person they both are. Thank you.

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Abstract

Switchgrass (*Panicum virgatum* L.) is a warm season perennial grass used widely as a forage crop. This research seeks to address improving biomass yield and predicted ethanol yield through certain traits by the following objectives: (1) differences in average biomass and predicted ethanol yields from leaves versus stems, (2) genetic variance and heritability estimates of biomass and ethanol yield traits (3) general (GCA) and specific combining ability (SCA) among the five parents for biomass and ethanol yield, and (4) correlations between agronomic and morphological traits. Five parents with varying morphological traits were crossed in a diallel design, excluding selfs. Clonal replicates of all crosses were planted at ETREC in Knoxville, TN and PREC in Crossville, TN.

The mean leaf biomass yield in 2012 was 22.9 g plant^{-1} [grams per plant] and 15.2 g plant^{-1} at two locations. In 2013 mean values were 41.5 g plant^{-1} and 57.9 g plant^{-1}. The mean stem biomass values were 29.4 g plant^{-1} and 15.6 g plant^{-1} in 2012. In 2013 the mean stem biomass yield increased to 84.6 g plant^{-1} and 98.6 g plant^{-1}. The average leaf to plant ratio in 2012 was 0.55 and 0.37 in 2013. The mean for predicted leaf ethanol in 2012 was 65.8 mg g^{-1} DM [milligrams per gram dry matter] and was 68.4 mg g^{-1} DM in 2013. Predicted stem ethanol mean was 61.67 mg g^{-1} DM.

GCA for leaf biomass ranged from -2.90 to 1.8 g plant^{-1}, and SCA values ranged from -5.7 to 7.1. GCA values -7.8 to 9.2 g plant^{-1} for stem biomass yield, and SCA values ranged from -10.9 to 11.0 g plant^{-1}. Predicted leaf ethanol yield GCA values ranged from -0.77 to 0.87, and SCA values ranged from -1.3 to 0.84. GCA values for predicted stem ethanol ranged from -3.2 to 2.6 mg g^{-1} DM, and SCA values from -1.2 to 1.3 mg g^{-1} DM. Narrow sense heritability estimates ranged from 0.03 to 0.23. Broad sense heritability estimates ranged from 0.16 to 0.6. High parent

heterosis was observed in all traits. There were correlations between agronomic traits and morphological traits.

Table of Contents

Chapter 1: Introduction……1

Chapter II: Literature Review……..3

Chapter III: Materials and Methods……9

a. Background Information on Plant Material……...9
b. Planting Details……10
c. Sample Harvesting and Processing……..10
d. Ethanol Analysis……..11
e. Morphological Trait Analysis……..12
f. Statistical Analysis……...12

Chapter IV: Results and Discussions……15

a. Leaf and Stem Biomass Yield……..15
b. Leaf to Plant Ratio……...19
c. Predicted Leaf and Stem Ethanol Yield……...20
d. General Combining Ability……..23
e. Specific Combining Ability……24
f. Genetic Variance……..25
g. Heritability Estimates……..26
h. High Parent Heterosis……..27
i. Correlations……..29
    a. Agronomic Traits ……29
    b. Morphological Traits……...29

Chapter V: Summary and Conclusions……..31

Literature Cited……..36

Appendix……41

VITA……..63

List of Tables

Table 1. Additive variance, dominance variance, phenotypic variance, narrow sense heritability (h^2), and broad sense heritability (H^2) for four yield components across 20 switchgrass crosses (including 10 reciprocals) located at Knoxville and Crossville, TN in 2012 and 2013……54

Table 2. High parent heterosis values for leaf and stem biomass and leaf to plant ratio across 20 switchgrass crosses and five parental lines at Knoxville and Crossville, TN in 2012 and 2013……55

Table 3. High parent heterosis values for predicted leaf and stem ethanol yield 20 switchgrass crosses and five parental lines at Knoxville and Crossville, TN in 2012 and 2013…….....56

Table 4. Correlation coefficients for among yield components of 20 switchgrass crosses (including ten reciprocals) located at Knoxville and Crossville, TN in 2012 and 2013……57

Table 5. Phenotypic correlation coefficients among yield components and morphological traits of 20 switchgrass crosses (including ten reciprocals) located at Knoxville and Crossville, TN in 2012 and 2013……58

Appendix A

Table.1A. Cross combinations of F_1 progeny including female, male, and reciprocal cross as well as parental lines derived from Alamo, Kanlow and Miami Varieties…….....60

Table.1B. Phenotypic Trait Measurements……61

List of Figures

Fig.1. Average leaf (A) and stem (B) biomass yield averaged across 20 switchgrass crosses for 2012 and 2013 at Knoxville and Crossville, TN……42

Fig.2. Bar graphs for leaf (A) and stem (B) biomass yield for 20 switchgrass crosses (including 10 reciprocals*) averaged across Knoxville and Crossville, TN locations for 2012 and 2013……43

Fig.3. Box plot of plant biomass yield of 20 switchgrass crosses (including ten reciprocals*) across Knoxville and Crossville, TN locations and two years (2012 and 2013)……...44

Fig.4. Box plot of leaf:plant biomass ratio averaged across Knoxville and Crossville, TN for 20 switchgrass crosses (including ten reciprocals*) in 2012 and 2013……. 45

Fig.5. Averaged predicted leaf (A) and stem (B) ethanol yield averaged across 20 switchgrass crosses for 2012 and 2013 at Knoxville and Crossville, TN…….46

Fig.6. Average predicted leaf (A) and stem (B) ethanol yield averaged across 20 switchgrass crosses (including ten reciprocals*) for Knoxville and Crossville, TN (A) across 2012 and 2013……47

Fig.7. Box plot for total predicted plant ethanol yield of 20 switchgrass crosses (including ten reciprocals*) across Knoxville and Crossville, TN locations and two years (2012 and 2013)……. 48

Fig.8. Plots for general combining ability (GCA) for leaf biomass yield (A), stem biomass yield (B), and leaf:plant ratio (C) for five switchgrass parental lines for 20 crosses across Knoxville and Crossville, TN and 2012 and 2013……..49

Fig.9. Plots for general combining ability for predicted leaf (A) and stem (B) ethanol yield of five switchgrass parental lines for 20 crosses (including ten reciprocals) at Knoxville and Crossville, TN in 2012 and 2013……50

Fig.10. Plots for specific combining ability (SCA) for leaf (A) and stem (B) biomass yield for 20 switchgrass crosses (including ten reciprocals*) across Knoxville and Crossville, TN in 2012 and 2013……51

Fig.11. Plots for specific combining ability (SCA) for leaf:plant ratio for 20 switchgrass crosses (including ten reciprocals*) across Knoxville and Crossville, TN in 2012 and 2013……52

Fig.12. Plots for specific combining ability (SCA) for predicted leaf (A) and stem (B) ethanol yield for 20 switchgrass crosses (including ten reciprocals*) across Knoxville and Crossville, TN in 2012 and 2013……53

Appendix A

Fig.1A. Map of F_1 population located at East Tennessee Research and Education Center, Holston Unit, Knoxville, TN…….....62

Fig.1B. Map of F_1 population located at Plateau Research and Education Center, Crossville, TN…….....63

CHAPTER 1

Introduction

Switchgrass (*Panicum virgatum* L.) is a perennial grass native to North America that has been used widely as a forage crop for livestock (Martinez-Reyna and Vogel, 2008). Switchgrass is separated into two ecotypes, upland and lowland, which are distinguished primarily on latitude and ploidy level. Upland switchgrass grows is drier areas, which experience drought (Casler, 2012). Lowland ecotypes are found in areas with wetter soils (Casler, 2012). According to Casler (2012), upland ecotypes are found most often north of 34° N latitude. Lowland ecotypes are more widely adapted, and are found in southern and coastal areas of the United States. The ploidy levels of switchgrass differ based on ecotype. The lowland varieties are generally tetraploid, while the uplands varieties are both tetraploid and octoploids (Casler, 2012).

In recent years, focus has shifted to using switchgrass as a biofuel crop due to the increased need for alternative fuel sources. The U.S. Department of Energy (DOE) funded research via the Bioenergy Feedstock Developed Program (BFDP) to assess feedstocks for potential use for bioenergy or bioproduct (McLaughlin and Kszos, 2005). A variety of herbaceous crops were evaluated and based on their ability to grow well on marginal land and compatibility with existing farming practices, switchgrass was chosen as a model crop for bioenergy (McLaughlin and Kszos, 2005). Resource inputs are generally modest, and because of its ability to grow on marginal land, farmers are able to utilize switchgrass in areas where high value agronomic crops may not be economically viable.

Most switchgrass research has been concentrated on evaluating it for forage production, which focused mainly on yield and quality. Breeding efforts for switchgrass as bioenergy have been limited. Thus, additional information on biomass yield and associated traits need to be

investigated. The objectives of this research were to determine i) Differences in average biomass yield and predicted ethanol yield from leaves and stems amongst 5 select crosses and their reciprocals ii) general (GCA) and specific combining ability (SCA) among the five parents for biomass yield traits and predicted ethanol yield traits iii) Genetic variance and heritability estimates of biomass yield traits and predicted ethanol yield traits, and iv) correlations between agronomic traits and morphological traits collected from 5 x 5 diallel.

CHAPTER II

Literature Review

Yield Factors

Switchgrass is a native perennial prairie grass used for livestock forage and soil and water conservation purposes. Yield of switchgrass varies depending on geography, ecotype, climate, soil condition, and management practices (Wullschleger et al., 2010). Timing of harvest is another important factor affecting yield, especially in multi-cut systems. 'Alamo' and 'Kanlow', two popular lowland varieties in the mid-south region have produced high biomass in either cutting system (McLaughlin and Kszos, 2005). Cutting prior to the first frost, before nutrient translocation also maximizes yield (McLaughlin and Kszos, 2005).

Upland and lowland varieties vary based on height and yield. It is estimated that lowland varieties generally produce more yield than upland varieties due to their larger height and tiller number (Wullschleger et al., 2010). Research suggests tiller size and density are better predictions of switchgrass yield than other factors in both space-planted and sward nurseries (Das et al., 2004). Large leaf width and higher tiller count have been related to the highest biomass yield (Das et al., 2004). In a study conducted by Boe and Beck (2008), it was found that the number of tillers was the best indicator for biomass production in space-planted nurseries. They suggested selecting for higher tiller number in a breeding program could be an indirect method of increasing biomass production. Furthermore they recommended that yield per tiller (average tiller weight), as seen in both switchgrass and alfalfa (*Medicago sativa* L.), could be a more useful criteria for yield estimation as opposed to only diameter of tillers and number of tillers. Larger individual tiller weight and lower tiller number produced more biomass than smaller individual tiller weight with higher tiller number.

Tiller (stem) length and diameter varies between ecotypes as well. Lowland ecotypes are generally taller and have thicker stems, while upland ecotypes tend to be shorter with thinner stems (Bhandari et al., 2011). Thus, yields vary between varieties within these ecotypes.

Height is also an indication of biomass potential for switchgrass plants. Talbert et al. (1982) found that taller plants usually weighed more and equated to more biomass produced per plant.

In addition, switchgrass is a perennial species that takes up to four years to reach its full biomass potential (Garland, 2008). In the first year of growth switchgrass will likely produce 30% of its total biomass yield. By the second year it is can produce 70%, and 100% of its full potential for total biomass by the third or fourth year (Garland, 2008).

Genetic Variation and Heritability

The ploidy level of switchgrass varies, with a chromosome number of x=9, n=18 (Casler, 2012). Switchgrass is either tetraploid or octoploid, which is largely based on ecotype. Lowland types are generally tetraploid whereas upland ecotypes can be either tetraploid or octoploid. However, octoploids tend to be two to three times more likely in upland ecotypes (Casler, 2012). Switchgrass is an obligate outcrossing species, and the percentage of selfed seed is less than 1%, leading to vast genetic diversity amongst populations (Casler, 2012). Cross-pollination is required due to gametophytic self-incompatibility (Rose et al. 2007). Plants within a variety are both heterogeneous and heterozygotic due to the high degree of out crossing. Due to the polyploidy and high degree of self-incompatibility switchgrass possesses high levels of allelic diversity amongst genotypes within populations (Bhandari et al., 2011). Thus, switchgrass possesses regionally adapted cultivars, largely due to the genotype by environment interactions

and natural selection. Crosses between compatible ploidy level varieties leads to genetically variable F_1 progeny within the same cross.

The use of a space-planted nursery allows for better observation of phenotypic and genotypic variation in switchgrass populations (Rose et al., 2007). This method of planting is not typical for forage plantings, but it has been shown to be an effective procedure to improve yield in other grass species.

Often traits such as plant height and tiller number are influenced by location, year, and the interaction of the two (Bhandari et al., 2011). In addition, biomass yield is affected by year and location, and their interaction (Bhandari et al., 2011). Thus, factors such as location, year, and weather conditions will play a key role in variation of traits in plant height, tiller number, and biomass yield.

Genetic Parameters and Mating Design

The use of a diallel design is unlike most switchgrass mating designs, which generally utilize the half-sib or full-sib mating designs. The use of a diallel allows breeders to assess the combining ability of the parental lines in the diallel (Sughroue and Hallauer, 1997). A diallel mating design allows breeders to examine the general combining ability (GCA) and specific combining ability (SCA) of parental lines of interest. General combining ability is used to assess the average performance of a line in hybrid combination (Griffing, 1956). Specific combining ability assesses specific cross combinations that perform better or worse than would be expected based on the average performance of the lines involved (Griffing, 1956). To achieve a reliable estimate of heritability and degree of dominance, a diallel containing a minimum of 8-10 parents has been suggested (Hayward, 1979). Griffing's Biometrical Analysis (Griffing, 1956) is a common

reference on the design and use of diallel mating in natural sciences. Utilizing a diallel mating design allows breeders to obtain genetic variances expressed in the F_1 of the crosses (Hayward, 1979). However, this will largely be based on the environmental conditions under which plants are grown and the parameters of interest.

Griffing's Biometrical Analysis provides four different methods to create and design diallel-mating designs (Griffing, 1956). The use of a full diallel analysis allows evaluation of all parents crossed in every combination including their reciprocals and selfs (Griffing, 1956). Method III was used for the purpose of this project, which includes a set of F_1's and reciprocals, but not the selfs. Genetic variance is evaluated using additive and dominant effects, σ^2_A and σ^2_D. In tetraploid plants, such as switchgrass, this can be estimated with the following:

$s^2_A = 4s^2_{gca} - 2/3s^2_{sca}$

$s^2_D = 6s^2_{sca}$ (Wricke and Weber, 1986).

Heterosis, or hybrid vigor, is defined as difference between the mean of the hybrid and the mean of the two parents (Martinez-Reyna and Vogel, 2008).Heterosis is reflective of the dominance variation and other non-additive genetic variation present.

Plant Composition and Leaf to Stem Ratio

Leaf to stem ratio has previously been used to help determine forage quality in grass species. In forage grass species, such as big bluestem (*Andropogon gerardi*) and Indiangrass (*Sorghastrum nutans* L.), knowing the individual leaf and stem yields provides researchers with an idea of dry matter yields. These yields help contribute to certain forage quality factors such as in vitro dry matter digestibility (IVDMD) content and crude protein, both essential for animal nutrition (Perry et al., 1979). For forage crops, such as alfala (*Medicago sativa*), leaf to stem ratio is

important for the amount of IVDMD, and traits associated with these have been subject to selection (Casler and Vogel, 1999).

Knowledge of leaf and stem components in bio-feedstock materials may lead to a better understanding of how to improve desired qualities. Examining the leaf to stem ratio of plant components, such as lignin, cellulose, and hemicellulose provides indicators for potential ethanol yield. Different switchgrass cultivars provide varying ethanol yields based on their lignin, cellulose and hemicellulose contents. Research indicates stems contain more lignin than leaves, thus creating problems during the fermentation process (David and Ragauskas, 2010). David and Ragauskas (2010) reported that the Kanlow cultivar contains more lignin in the stems, as well as cellulose and hemicellulose. Lignin was found in lesser amounts in the leaves, although cellulose and hemicellulose were lower as well. Breeding efforts and molecular research continue to evaluate ways to reduce lignin and increase cellulose and hemicellulose (David and Ragauskas, 2010).

According to Butkute et al. (2013), the composition of lignin and carbohydrates varies in leaf and stem partitions of switchgrass plants, which can imply potential of use for bioenergy production. Stems of switchgrass are said to be more lignified than leaves, and can be used for liquid and solid biofuel production. The stage of harvest plays a key role in the availability of ethanol, and the optimum growth stage to maximize ethanol, and harvest generally recommended at the seed filling stage (Butkute et al., 2013)

Ethanol Content in Switchgrass

Lignocellulosic ethanol can be produced from cellulose and hemicellulose using saccharification and fermentation processes (David and Ragauskas, 2010). Cellulose, hemicellulose, and lignin

are all concerns for the conversion process, mainly cellulose and hemicellulose. Lignin is also a major plant component, which gives the plant its structure and rigidity. However, unlike cellulose and hemicellulose, lignin cannot be easily broken down in conventional ethanol fermentation processes, reducing the potential ethanol yield available (David and Ragauskas, 2010). However, lignin can be used for other biofuel sources, such as bio-oil. Reducing lignin in switchgrass plants is a major target for biofuel breeding efforts.

Results from near infrared reflectance spectroscopy (NIRS), commonly used to evaluate forage quality of various plant species, may be used to estimate ethanol yield using calibrations developed by Vogel et al. (2010). Compared to wet chemistry procedures, NIRS is an inexpensive technology that provides accurate results for ethanol yield relatively quickly. NIRS calibrations for woody and herbaceous biofuel feedstocks include ash, lignin, uronic acid, cell wall sugars, C, H, N, O and the predicted ethanol content (Vogel et al., 2010).

CHAPTER III

Materials and Methods

Background Information on Plant Material

In 2010, five parent lines (A1, T1, K2, K3, and M) were chosen based on yield, tiller number, height, stem size, leaf size, leaf color, and leaf orientation. The parental lines were selected from 'Alamo' (A1, T1) and 'Kanlow' (K2, K3) varieties, plus a selection of the Miami (M; PI 421901) variety, all of which are lowland ecotypes.

In January of 2011, the selected parental lines were divided into clonal propagules. These plants were transplanted from the field to 7.6-liter pots, and placed in a glass house at the University of Tennessee, East Tennessee Research and Education Center, Knoxville. Plants were initially fertilized with Osmocote™ fertilizer at a rate of 50 kg ha^{-1} N. A monthly fertilization rate of 20 N-20 P_2O_5 -20 K_2O (3.7 mL per pot) was then applied.

A complete set of diallel crosses was made among the five parents (including reciprocals, but excluding selfs). For each cross combination, a single tiller from each parent was placed in a pollination bag. When the stigma was visible at the tip of the panicle, tillers were placed in a bag. Every two days the bags were shaken lightly to distribute the pollen amongst the tillers. Once pollination was complete, panicles were bagged separately to allow seed ripening when the stigma had visibly desiccated.

Crossed seeds were harvested and approximately 36 seedlings and the reciprocal combination from each cross were randomly selected for propagation. Furthermore, a total of 304 seedlings were chosen for the borders in the space-planted nurseries. Eight tillers were grown from harvested seed in conetainers (Stuewe & Sons, Corvallis, OR). The F_1 progeny from the eight

tillers were later divided into three clonal propagules, and the parental plants were divided into 15 propagules. Plants remained in the glass house until they were ready to be transplanted into the field. In total, twenty clonal propagules of each cross were produced and twenty clonal replicates of each parental line were distributed randomly amongst the four blocks at each location.

Planting Details

In June of 2012 the propagules were transplanted to a field site at the East Tennessee Research and Education Center- Holston Unit (ETREC) in Knoxville, TN (35º58'11.3"N 83º51'08.7"W, soil type: Shady-Whitewell complex) and Plateau Research and Education Center (PREC) in Crossville, TN (36º00'49.7"N 85º07'57.7"W, soil type: Lonewood loam). The Shady-Whitewell complex is described as a sandy clay loam found in low terrace areas, and is well-suited for crop land, hay, or pasture. The Loneweood loam is a fine loamy soil found on ridges. These two locations were laid out using a randomized complete block design, with each field containing four blocks. Throughout the entire field there were 20 replications (F_1 plants) of each cross, and 20 clonal parental replicates. Plants were spaced 1 m apart. Soil samples were taken prior to planting to determine necessary fertilization rates for each location.

Sample Harvesting and Processing

In fall 2012, the space planted nurseries at both ETREC and PREC were harvested between late October and early November. Plants at ETREC and PREC locations were harvested using small hand shears, cut 15 cm above the base of the plant to avoid injury to the plant's crown. At each location, the entire plant biomass weight was collected. In 2012, the entire plant was sampled due to its small size, and weighed for wet weight. Samples were dried in a batch oven

(Wisconsin batch oven, Wisconsin Oven, East Troy, Wisconsin) at 60 °C for 48 to 72 hours and then reweighed.

In the fall of 2013, due to the increased size, plants were harvested using a sickle bar mower 15 cm above the ground to avoid damaging the plant's crown and growing points. A sample of approximately ten tillers was collected; wet weight was recorded, and then dried in the batch oven at 60 °C for 48 to 72 hrs. and then reweighed.

Plants were separated into leaves (including leaf sheath) and stems (including panicle and seed) and weights were recorded for stems and leaves for each sample. Dried samples, stems and leaves separately, were ground using the Wiley Laboratory Mill (Thomas Scientific, Swedesboro, NJ) to pass through a 1 mm screen. Smaller samples were processed using the Cyclone grinder, also with a 1 mm mesh screen (UDY Cyclone Sample Mill, UDY Corporation, Fort Collins, Colorado).

Ethanol Analysis

The ground stem and leaf samples were analyzed using Near Infrared (NIR) Spectroscopy (Foss NIRSystems 6500 Feed & Forage Analyzer with Sample Transport Reflectance Only, Foss Analytical, Hilleroed, Denmark). A global H statistical test was used to check the current data fit against the spectral profile (Murray and Cowe, 2004). A 'mixed hay' equation was used to determine the amounts of lignin, ADF, and NDF in the stem and leaf samples. Lignin, acid detergent fiber (ADF), and neutral detergent fiber (NDF) were also evaluated.

An equation developed by Lorenzo et al. (2009) for corn stover ethanol was used to predict the ethanol yield in leaf and stem samples based on NDFD48 (neutral detergent fiber digestibility at 48 hours) and NDF (neutral detergent fiber). This equation was designed to estimate ethanol

yield from SSF with corn stover. However, from previous switchgrass studies using NIRS, it explained 97.5% of the variation in predicted ethanol yield (Sykes, Ph.D. Dissertation, 2014). The equation is as follows:

$$-0.114 + 0.00229(NDFD) + 0.00117(NDF) = \text{ethanol yield (Lorenz et al., 2009)}$$

Morphological Trait Analysis

Plant heights were taken prior to harvest in the fall each year and in the spring of 2013 and 2014 (due to fall planting in 2012). In addition, plant tillers were counted and leaf and stem diameter measurement were taken in the spring of the 2013 and 2014 growing seasons. These morphological traits were used to correlate to the agronomic traits (leaf to plant ratio, leaf biomass, stem biomass, predicted leaf ethanol, and predicted stem ethanol) analyzed in this study.

Statistical Analysis

Statistical analyses were performed using SAS statistical software v. 9.3 (SAS Institute, Cary, NC). The traits leaf to plant ratio, leaf biomass, stem biomass, predicted leaf ethanol, and predicted stem ethanol were analyzed using BLUP (Proc Mixed) to predict GCA and SCA values. The GCA and SCA values were used to derive the additive and dominance variance. From these values, heritability estimates were calculated. When using BLUP analysis, year, location, block, and their interactions are considered fixed effects, while cross and line are placed as random effects. In order to determine if crosses differed from one another for individual traits, Analysis of Variance (Proc Mixed) was used by designating cross as a fixed effect and year and location as random. Reciprocal effects were analyzed using contrasts between F_1 progeny crosses and their reciprocals. For example, A1xT1 and T1xA1 were compared based on the yield or

value of a desired trait to determine if significant differences were apparent between reciprocal crosses. Pearson's correlations (Proc Corr) were used to calculate correlation coefficients between desired traits. In addition to agronomic yield, leaf and stem morphological trait data were also collected and analyzed. Pearson correlation coefficients were calculated between morphological traits (tiller number, plant height, tiller diameter, leaf diameter) and shoot correlations (leaf biomass, stem biomass, leaf to plant ratio, predicted leaf ethanol, and predicted stem ethanol). Also correlation coefficients were calculated between morphological traits and total plant biomass and total predicted plant ethanol. Tiller number, height, leaf diameter, and stem diameter were collected in the spring 2013 and 2014 at both locations. Height was also taken in the fall for 2012, 2013, and 2014 prior to harvesting.

The statistical model showing male, female and male*female effects are as follows:

$y_{ijlnp}=\mu+B_j+M_l+F_n+MF_{ln}+R(MF)_{p(ln)+}e_{ijlnp}$

y_{ijlnp}= observed value of a given character for an individual plant

μ= overall mean

B_j= effect of block *j*

M_l= effect of male *l, l=1-8*

F_n= effect of female *n, n= 1-8*

MF_{ln}= interaction between male *l* and female *n*

$R(MF)_{p(ln)=}$ effect of rep within family, *p*

Assuming a normal distribution, the variance terms should be as follows (Becker, 1984):

$$\sigma^2_m = \sigma^2_f = 1/4\ \sigma^2_a + 1/16\ \sigma^2_{aa} + \ldots$$

$$\sigma^2_{mf} = 1/4\ \sigma^2_d + 1/8\ \sigma^2_{aa} + 1/16\ \sigma^2_{dd} \ldots$$

$$\sigma^2_{c\,(m*f)} = 1/2\ \sigma^2_d + 3/4\ \sigma^2_a + 7/8\ \sigma^2_{ad} + 15/16\ \sigma^2_{dd} \ldots$$

Genetic Variances:

$$\sigma^2_a = 4\ \sigma^2_{gca} - 2/3\ \sigma^2_{sca}$$

$$\sigma^2_d = 6\ \sigma^2_{sca}$$

$$\sigma^2_a = 2(\sigma^2_m + \sigma^2_f) = 1/4\ \sigma^2_a + 1/16\ \sigma^2_{aa} + \ldots$$

$$\sigma^2_d = 4(\sigma^2_{mf}) = \sigma^2_d + 1/2\ \sigma^2_{aa} + 1/2\ \sigma^2_{ad} + 1/4\ \sigma^2_{dd} \ldots$$

$$\sigma^2_i = \sigma^2_{r\,(m*f)} - (\sigma^2_m + \sigma^2_f) - 3\ \sigma^2_{mf} = 1/4\ \sigma^2_{aa} + 1/2\ \sigma^2_{ad} + 3/4\ \sigma^2_{dd} \ldots$$

Narrow sense heritability:

$$h^2 = \sigma^2_a / \sigma^2_p$$

Broad Sense heritability:

$$H^2 = (\sigma^2_a + \sigma^2_d) / \sigma^2_P$$

Where σ^2_P is the sum of the additive, dominance, and residual components.

<u>Heterosis:</u>

Values for high-parent heterosis were evaluated for each assessed trait. These were calculated using the difference between progeny means and high parent mean values.

High-parent (HP) heterosis:

((F_1 progeny mean - high parent mean)/high parent mean)= HP heterosis

CHAPTER IV

Results and Discussion

Leaf and Stem Biomass Yield

Crosses did not differ locations for leaf biomass; however, year had a significant effect on leaf biomass of crosses ($P<0.05$; Fig. 1A). In 2012, the overall mean leaf biomass for all crosses in Knoxville was 22.5 g plant $^{-1}$ compared to 15.2 g plant $^{-1}$ in Crossville (Fig. 1A). This increased in 2013 to 41.5 g plant^{-1} in Knoxville and 57.9 g plant^{-1} in Crossville (Fig. 1A).

Individual crosses differed significantly for leaf and stem biomass ($P<0.05$). In 2012, K2xA1 (cross of Kanlow x Alamo lines) had the highest leaf biomass (28.8 g plant^{-1}) and K2xK3 had the lowest mean leaf biomass (Fig. 2A). As this was the establishment year the leaf biomass values were lower than the second year. In 2013, means for individual crosses increased as plants continued to increase in production capacity. The top performing crosses in 2013 for combined locations were K2xM (cross of Kanlow x Miami lines) (71.9 g plant^{-1}) and T1xM (cross of Alamo x Kanlow lines) (67.0 g plant^{-1}; Fig. 2A). Again, the cross producing the lowest mean leaf biomass was K2xK3 (Kanlow lines) (35.4 g plant^{-1}; Fig. 2A). This particular cross produced the lowest amount of mean leaf biomass in both years. Across both years and locations K2xM (47.6 g plant $^{-1}$) and 'T1xM' (47.1 g plant $^{-1}$) had the largest leaf biomass and K2xK3 had the lowest mean leaf biomass (21.7 g plant^{-1}; Fig. 2A). These two particular crosses displayed reciprocal effects resulting in higher leaf biomass yield across both years and location when Miami was used as the male parent. In general, the crosses with the least leaf biomass across both years and locations contained Kanlow as the maternal parent.

There were significant reciprocal effects across both years and locations for leaf biomass ($P<0.05$). For example, when averaged across locations and years, the cross A1xK3 was 4.94 g

plant^{-1} higher than the mean leaf biomass for cross K3xA1. Cross K2xM was 5.74 g plant^{-1} higher than the mean leaf biomass for its reciprocal MxK2 (Fig. 2A). Lastly, T1xM was 6.3 g plant^{-1} higher than the mean leaf biomass for its reciprocal MxT1 when averaged across locations and years (Fig. 2A). Thus, among these crosses there was some evidence of maternal effects on leaf biomass. These results support a study by Bhandari et al. (2014) in which biomass yield was shown to be influenced by reciprocal effects.

There were significant differences between 2012 and 2013 for stem biomass ($P<0.05$). The overall mean stem biomass for all crosses at Knoxville in 2012 was 29.4 g plant^{-1} (Fig. 1B). In 2013, the overall mean stem biomass for all crosses at Knoxville increased to 84.6 g plant^{-1} (Fig. 1B). Similarly, at Crossville in the overall mean for stem biomass increased from 15.6 g plant^{-1} to 98.6 g plant $^{-1}$ from 2012 to 2013 (Fig. 1B). As plants reached their second year of maturity, the stem biomass yield increased by approximately 68 g plant $^{-1}$ when averaged across 20 crosses. On the other hand, locations did not differ for stem biomass within or between years ($P>0.05$). However, there were significant differences in location by year interactions ($P<0.05$) because the mean stem biomass was higher ($P<0.05$) in Knoxville than Crossville in 2012, but the reverse was true in 2013 (Fig. 1B).

In 2012, leaf biomass and stem biomass values did not differ significantly from each other ($P>0.05$); however, in 2013, stem biomass was significantly larger than leaf biomass ($P<0.05$; Fig. 1A and B). Butkute et al. (2013) noted that switchgrass stems contained higher biomass yield, and would be suitable for direct combustion and liquid biofuel production. This was reaffirmed by Frank et al. (2004) in which both switchgrass cultivars examined produced more stem biomass than leaf biomass. The increased stem biomass is likely caused by an increase in more lignin than leaves (Butkute et al., 2013). Furthermore, the biomass yields in 2013 are likely

more indicative of plants when they are approaching their maximum production potential at yr. 3-4 (Garland, 2008).

In 2012, the crosses producing the largest amount of stem biomass were K2xA1 (42.2 g plant $^{-1}$) and the reciprocal A1xK2 (41.3 g plant $^{-1}$; Fig. 2B). The lowest performing cross in 2012 across both locations was MxK3 (7.8 g plant $^{-1}$) as well as its reciprocal K3xM in 2013 (Fig. 2B). This cross had largest leaf to plant ratio (0.75), which resulted from a lower leaf biomass. As previously indicated, the Miami parent is a robust and leafy plant, resulting in lowered stem biomass and higher leaf biomass. In addition, this was the first year after establishment and yields were typically lower than yields during the second year. In 2013, across both locations, A1xK2 had the highest average stem biomass (126.2 g plant $^{-1}$; Fig. 2B). Across both years and locations the highest stem biomass was A1xK2 (83.9 g plant $^{-1}$), while K3xM was the lowest again (34.3 g plant $^{-1}$; Fig. 2B).

As was true with leaf biomass, there were significant reciprocal cross effects across both years and locations for stem biomass ($P<0.05$). The cross A1xK3 was 8.2 g plant $^{-1}$ higher than the reciprocal K3xA1. In addition, K2xT1 had a mean stem biomass weight 6.2 g plant $^{-1}$ higher than its reciprocal T1xK2 (Fig. 2B). There were reciprocal effects from the cross K2xM resulting in an increase of 11.0 g plant $^{-1}$ from the reciprocal cross MxK2. Lastly, T1xM was 12.7 g plant $^{-1}$ higher than its reciprocal MxT1(Fig. 2B). The positive increase in mean stem biomass indicates there were maternal effects for stem biomass among these crosses. These results indicate when the Miami M parent is used as the female plant in a cross it could result in a decreased mean stem biomass but an increased leaf biomass.

There were significant differences among crosses for overall biomass yield across both years and locations ($P<0.05$). The top performing cross for Knoxville and Crossville in 2012 and 2013 was 'T1xM' (cross of Alamo x Kanlow lines) (Fig. 3). Crosses K3xM and K2xK3 were the poorest performing crosses and did not differ significantly from one another ($P>0.05$; Fig. 3). As seen in Fig. 3, there are many outliers. Many of these outliers are 2013 data points that exceeded the overall population mean of years combined. The top performing individual clone was K2xT1-17, which had an overall biomass of 5138.6 g plant^{-1} in 2013 (data not shown). In addition, this plot also had predicted leaf and stem ethanol yield, as well as overall predicted ethanol values that exceeded the means for 2013, and years combined. These high values from outliers may be due to exceptional plants or to environmental effects such as lack of competition or errors in the data. Locations did not differ significantly ($P>0.05$). However, years differed significantly for overall biomass yield ($P<0.05$). Due to the plants' age and developmental stage, biomass yield in 2012 was less than in 2013. This is to be expected based on the age of the plants, which will continuously increase biomass as they approach maturity around yr. 3 or yr. 4. Across both locations and years, there were significant differences among crosses ($P<0.05$; Fig. 3). The cross T1xM was the highest performing cross (Fig. 3). In addition, in both of these years K2xK3 and its reciprocal K3xK2, crosses MxK2, MxK3, MxT1, T1xK2 and T1xK3 were the poorest performing crosses overall (Fig 3). Theses crosses displayed low yields and low distributions but had several outliers. In three of the crosses and their reciprocals (K2xT1/T1xK2; K3xT1/T1xK3; MxT1/T1xM) there were fairly large differences in plant biomass yields, indicating that there appears to be maternal effects on plant biomass yield (Fig. 3).

Leaf to Plant Ratio

Leaf to plant ratio is the ratio of leaves in comparison to the entire biomass of the plant, excluding the root system. The cross means for leaf to plant ratio differed ($P<0.05$) between years and locations but not when averaged across both locations. The interaction of cross by location and cross by year were not significant, thus the distributions of crosses were computed across locations and years (Fig. 4). In 2012, the overall mean for all crosses for leaf to plant ratio was 0.55; whereas the ratio was 0.37 in 2013 (Fig. 4). Plants in the first year were still in their juvenile state, and generally produced more leaf biomass than stem biomass, accounting for a higher leaf to plant ratio in 2012. According to Garland (2008), switchgrass produces nearly 70% of its overall biomass yield by the second year and 90-100% by the third year. Thus, more stems were produced during the second year (2013), reducing the leaf to plant ratio by 18% from 2012 to 2013.

At Knoxville in 2012 crosses MxK2 (0.72) and MxK3 (0.67) had the largest leaf to plant ratio (Fig. 4). The individual plot MxK2-2 had a leaf to plant ratio of 0.75, which is 0.20 above the mean in 2012 (DNS). However, the overall biomass yield of this plant was lower than the population mean. Crosses at Knoxville had a lower leaf to plant ratio in both years, indicating a greater stem biomass at this location. In 2013, MxK3 (0.45), MxT1 (0.42), MxK2 (0.40), and T1xM (0.40) exhibited the largest leaf to plant ratios; whereas K2xT1 had the lowest leaf to plant ratio (0.28) (Fig. 4). Frank et al. (2004) exhibited similar results with cultivars with stems that produced 48-56% of the total biomass of above ground switchgrass plants. The individual clone A1xK3-19 (0.74) far exceeded the overall population mean for 2013 (DNS). In addition, this clone had an overall biomass yield greater than the overall population mean.

At Crossville, in 2012 crosses MxK3 (0.75), MxT1 (0.68), and MxK2 (0.66) displayed the largest leaf to plant ratio. These three crosses all contain a Miami parent, which is known for its large leaves and could be contributing to a higher leaf to plant ratio. In 2013 at this location again two of these crosses MxT1 (0.51) and MxK3 (0.48) had the highest leaf to plant ratio (Fig. 4). The decrease in this value is likely due to the increase in stem material as the plant continues to mature.

There were significant reciprocal effects across both years and locations for leaf to plant ratio. The cross A1xT1 had an increase of 0.02 in leaf to plant ratio compared to the reciprocal T1xA1, indicating that when A1 is used as the female parent in the cross there were maternal effects for that cross. Three crosses/reciprocals (K2xT1/T1xK2; K3xT1/T1xK3 and MxT1/T1xM) exhibited a 0.04 decrease in the leaf to plant ratio. The data from these crosses indicates that there were maternal effects on leaf to plant biomass ratio.

Predicted Leaf and Stem Ethanol

For the predicted leaf ethanol trait there were no significant differences ($P>0.05$) between years; however there were significant differences ($P<0.05$) between the two locations (Fig. 5A). In 2012, the overall population mean for combined locations was 65.8 mg g^{-1} DM. The overall population mean for combined locations in 2013 was 68.4 mg g^{-1} DM. Plants in Knoxville were predicted to produce more leaf ethanol than those in Crossville in 2013 but not 2012 . Since the clones were identical at both locations, this means that environmental conditions have an effect on the tissue composition that affects ethanol yield. According to Esbroeck et al. (1997), there is a strong relationship between the temperature and leaf appearance rate. With the two varying climates and environments, this could explain the increase in predicted leaf ethanol between the

two locations in 2013 based on the developmental stages of the leaf. There was also a significant interaction between location and year ($P<0.05$). Across both years, there was not a significant interaction between Knoxville and Crossville. In addition, from 2012 to 2013, leaf ethanol values increased significantly in Knoxville by 5.5 mg g^{-1} DM ($P<0.05$) (Fig. 5A).

At Knoxville and Crossville across both years, the cross producing the highest predicted leaf ethanol was T1xK3 (cross of Alamo x Kanlow lines) (73.0 and 70.0 mg g^{-1} DM respectively; Fig. 6A). The lowest producing cross for predicted leaf ethanol was K3xM (Kanlow and Miami lines) (65.0 mg g^{-1} DM). In Crossville across both years T1xK3 also produced the largest amount of predicted leaf ethanol (70.0 mg g^{-1} DM; Fig. 6A). However, AlxK2 (Alamo and Kanlow lines) produced the least amount of predicted leaf ethanol (61.6 mg g^{-1}DM). Across both years and locations there were no significant reciprocal effects shown for leaf ethanol ($P>0.05$).

In 2012 and 2013, the values for predicted leaf vs. stem ethanol did not differ significantly ($P>0.05$). Stem ethanol content did not vary by location or year ($P>0.05$). However, there was a significant interaction between location and year ($P<0.05$). In 2012, the overall cross means at Crossville and Knoxville differed by 2.12 mg g^{-1} DM predicted stem ethanol yield ($P<0.05$) (Fig. 6B).

Averaged across both years and locations the highest performing crosses for predicted stem ethanol were T1xK3 (67.1 mg g^{-1} DM) and T1xK2 (66.1 mg g^{-1} DM; Fig. 6B). Although not significant ($P>0.05$), it is important to note that at each location, Knoxville and Crossville, that in addition to producing the most predicted leaf ethanol the cross T1xK3 also produced the largest amount of predicted stem ethanol (Fig. 6B). Thus, in terms of ethanol production, cross T1xK3 is the top producing cross. Cross A1xK2 had the lowest amounts of predicted stem ethanol across

both locations and years. The individual clone K3xK2-14 at Crossville in 2012 had a predicted stem ethanol yield of 77.3 mg g^{-1} DM (DNS) and had a predicted leaf ethanol yield of 90.4 mg g^{-1} DM. It should be noted that in most cases the clones with higher predicted stem and leaf ethanol yields did not have overall biomass yields that exceeded the overall population mean. For example, the K3xK2-14 clone only produced 6.9 g $plant^{-1}$ of plant biomass yield. The individual clone A1xM-7 in Crossville in 2012 had an overall biomass yield of 95.4 g $plant^{-1}$, predicted leaf ethanol yield of 72.4 mg g^{-1} DM, and predicted stem ethanol yield of 66.5 mg g^{-1} DM. Both the predicted leaf and stem ethanol yields exceeded the overall population means for each trait. Averaged over crosses, the leaf ethanol and stem ethanol values do not differ significantly across years and locations. This is supported by the overall predicted plant ethanol values, where T1xK3 is also the highest producing cross (68.5 mg g^{-1} DM; Fig. 7). There were no significant reciprocal effects observed for the stem ethanol trait across both years and locations ($P>0.05$).

The predicted plant ethanol values were calculated based on the leaf to plant ratio of the cross. Individual crosses differed significantly for predicted plant ethanol yield ($P<0.05$) across both years and locations. There were no significant differences between years or locations ($P>0.05$). The amount of predicted plant ethanol yield stayed consistent across years with little variation among cross means (Fig. 7). The cross T1xK3 had the highest predicted whole plant ethanol yield (68.5 mg g^{-1} DM; Fig. 7), while A1xK2 had the lowest amount (Fig. 7). In 2012, T1xK3 was also the top performing cross and in 2013, T1xK3 and T1xK2 had the largest amount of predicted plant ethanol yield (Fig. 7).

General Combining Ability

The general combining ability (GCA) of a parental line provides the deviation of progeny performance of a parental line in all cross combinations with a chosen set of parents, compared to the overall average progeny of the diallel set of crosses. The parental line chosen from Miami (M) had the largest GCA value (1.8 g plant^{-1}) for leaf biomass, and line K3 had the smallest (-2.90 g plant^{-1}; Fig 8A). Line A1 had the highest GCA value (9.18) across both years and locations for stem biomass (Fig. 8B). Conversely, line K3 had the lowest GCA value (-7.83 g plant^{-1}; Fig. 8B). The line K3 had the lowest GCA values for both leaf and stem biomass yield, indicating this line does not perform well in comparison to other parental lines used in this study. In addition, GCA values for stem biomass yield tended to be larger than those for leaf biomass yield. This could be due to larger variation of stem biomass yield for parental lines.

Considering leaf to plant ratio across both years and locations, line M, a PI from Florida, had the highest GCA value (0.05); while K2, a Kanlow derived line, had the lowest GCA value (-0.004; Fig. 8C) for leaf to plant ratio. The M parental line is a very leafy and robust plant that resulted in a high leaf to plant ratio, thus it had the highest GCA value for leaf to plant ratio.

For predicted leaf ethanol yield line T1 had the highest GCA values (0.87 mg g^{-1} DM; Fig) and line K2 had the lowest GCA value for leaf ethanol content (-0.77 mg g^{-1} DM; Fig. 9A). Line T1 produced the greatest GCA values for predicted stem ethanol yield (2.57 mg g^{-1} DM; Fig. 9B); whereas, Line A1 had the lowest GCA value (-3.21 mg g^{-1} DM; Fig. 9B). The parental line T1 appeared in the top performing crosses for all predicted ethanol traits (leaf, stem and whole plant) indicating this would be a potential line for crossing purposes in the future. However, line T1 did not perform as well for leaf and stem biomass yields, in which it was negative in both cases. Thus, the parental line T1 would be best suited for ethanol yield.

Specific Combining Ability

Specific combining ability (SCA) indicates which crosses perform better or worse than the average performance of the entire diallel set of crosses. For leaf biomass yield cross T1xM had the greatest SCA value (7.10 g plant^{-1}) and cross K2xK3 had the lowest SCA value (-5.74 g plant^{-1}) for leaf biomass across both years and locations (Fig. 10A). Furthermore, cross T1xM had the highest SCA value for stem biomass (11.03 g plant^{-1}) across both years and locations (Fig. 10B). This large value provided evidence that cross T1xM performed well above the population mean. On the other hand, its reciprocal cross MxT1 had the lowest SCA value (-10.94 g plant^{-1}; Fig.10B). There was a significant reciprocal effect between these two crosses indicating maternal effects. The cross T1xM had the highest SCA values for both leaf and stem biomass yield, indicating this cross could result in higher biomass yields of each of these traits. This is the case for leaf biomass yield, where T1xM was one of the top performing crosses across both years and locations and T1xM produced the highest overall biomass yield. However, T1xM was not a top performing cross for stem biomass yield across both years and locations, although did perform above the population mean for stem biomass yield for combined years and locations.

For leaf to plant ratio cross MxK3 had the highest SCA value (0.039), while T1xM had the lowest SCA value (-0.035; Fig. 11). In addition, MxK3 had the highest leaf to plant ratio cross means. With the parental lines M and K3 having the highest GCA values, the combination of these two likely yielded high leaf to plant ratio values. The cross T1xM had the highest SCA values for stem biomass yield, and thus indicates a lowered SCA value for leaf to plant ratio. General and specific combining ability values for leaf to plant ratio are relatively small, indicating that the F_1 progeny from the cross combinations of these parental lines performs very

similarly. Most combinations when Kanlow was used as the female parent had negative SCA values for leaf to plant ratio is indicating Kanlow does not combine well for this trait.

The cross T1xK3 had the best SCA value for predicted leaf ethanol yield (0.84 mg g^{-1} DM), while cross K3xM had the lowest (-1.25 mg g^{-1} DM; Fig. 12A). For predicted stem ethanol yield, cross K2xM performed the best (1.29 mg g^{-1} DM), while cross A1xK2 had the lowest values (-1.24 mg g^{-1} DM; Fig. 12B). In general, crosses using the parental line K3 as the female parent produced negative GCA value. Both of the parental lines T1 and A1 had more positive SCA values than negative (Fig.12).

Genetic Variance

Amongst the observed traits there was a large amount of genetic variation. Genetic variance values, whether additive or non-additive, help choose the type of selection would be effective in the future for this diallel population. A higher additive variance than dominance would be best suited for recurrent selection. This breeding method is used in both self-pollinated and cross-pollinated species, and typically exploits the use of high heritability estimates and additive variance (Acquaah, 2007). However, due to the lower heritability estimates and additive variance of this diallel population, recurrent selection would not be ideal.

Due to the larger dominance variance in most of the traits associated with these crosses synthetic or hybrid breeding methods might be advisable for these crosses. Leaf biomass yield had a phenotypic variance of 503.05. In this case there was nearly tenfold more dominance variation than additive (Table 1). Stem weight also had high phenotypic variance (1629.83). Again, there was more dominance than additive variation. Predicted leaf ethanol yield had similar additive (3.03) and dominance (3.96) values, contributing to a lowered phenotypic variance in

comparison to other traits. Predicted stem ethanol yield had about 1.6 times more dominance (30.56) than additive (19.69). Casler and Vogel (2014) describe switchgrass as having a high degree of outcrossing, high levels of heterogeneity, and high levels of heterozygosity leading to large amounts of genetic variation within a population. These factors, as displayed here and the presence of more dominance variation in this diallel population, and occurrences of high parent heterosis for certain traits indicate that using hybrid breeding methods could be beneficial.

Heritability Estimates

Heritability estimates were calculated using the additive and dominant values derived from GCA and SCA values for line and cross. These values were used in combination with error variance to compute the narrow sense (h^2) and broad sense heritability (H^2) for desired traits.

Leaf biomass had a very low narrow sense heritability estimate of 0.03 across both years and locations (Table 1). This was not unexpected because the variance estimates are among F_1 clones and not among F_2's or a later generation segregating population. The broad sense heritability estimate was much larger due to the contribution of dominance variance (Table 1). The heritability estimates for stem biomass were 0.11 for narrow sense heritability and 0.32 for broad sense heritability (Table 1). Again, the large amount of dominance variation contributed to the total genetic variation, resulting in a higher broad sense heritability estimate.

Predicted leaf ethanol narrow sense heritability estimates were very low (0.07), with a larger broad sense heritability of 0.16 (Table 1). Additive (3.03) and dominance (3.96) variation were very similar in magnitude for predicted leaf ethanol. Predicted stem ethanol across both years and locations had a moderate narrow sense heritability estimate of 0.23 and a broad sense

heritability estimate of 0.60 (Table 1). This value is fairly large, and was caused by a larger portion of dominance variance.

High Parent Heterosis

Parent yields for leaf biomass tended to be lower than the F_1 progeny across both years and locations. The overall F_1 mean for all crosses was 33.90 g plant^{-1}. There were five crosses that had significant high parent heterosis ($P<0.05$) for leaf biomass yield (Table 2). There were two Kanlow x Alamo combinations, and two Kanlow x Miami combinations. The Kanlow x Miami combinations were reciprocal crosses of one another. There was one Alamo x Miami combination.

The F_1 progeny for stem biomass yielded were superior to the performance to the parental lines A1, K2, K3, M, and T1. There were four significant crosses which exhibited high parent heterosis for stem biomass yield ($P<0.05$) (Table 1). Of these four two were Alamo x Kanlow combinations, one Kanlow x Miami combination, and one Alamo x Miami combination for this trait. In this case, the combination of two Alamo x Kanlow combinations indicates that it is more likely to see increased stem biomass when crossed with these parental lines.

For leaf to plant ratio across both years and locations, parents exhibited values fairly close to means of the F_1 progeny. For example, the means for leaf biomass for the five parental lines were 0.44, 0.43, 0.41, 0.63, and 0.43 g plant^{-1} for A1, K2, K3, M, and T1 respectively. There were six crosses with significant high parent heterosis for leaf to plant ratio ($P<0.05$) (Table 1). Of these crosses, there were four Kanlow x Miami combinations. These crosses were reciprocals of one another. The Miami line is known for its large and numerous leaves. This is likely the reason for larger number of Miami combinations producing higher leaf to plant ratios than the

other parents. In addition, there was an Alamo x Kanlow combination, and one Alamo x Miami combination.

The parent values for predicted leaf ethanol tended to fall close to the mean values for the progeny across years and locations. For example, the means for the five parental lines were 65.8, 61.8, 66.2, 66.7, and 69.2 mg g^{-1} DM for A1, K2, K3, M, and T1 respectively. The overall F_1 mean for predicted leaf ethanol yield for all crosses was 67.1 mg g^{-1} DM. There were 3 significant crosses for high parent heterosis for this trait ($P<0.05$) (Table 3). Three of these crosses were Alamo x Kanlow combinations. Three crosses were Miami x Kanlow combinations. Two of these were Kanlow x Kanlow combinations.

The parent values for predicted stem ethanol were similar to the mean values of the progeny across both years and locations. For example, the means of the five parental lines were 56.6, 59.3, 62.8, 57.2, and 64.4 mg g-1 DM for Al, K2, K3, M, and T1 respectively. The overall F_1 means for predicted stem ethanol for all the crosses was 61.71 mg g^{-1} DM. Of these 20 crosses there were 3 that had significant high parent heterosis for predicted stem ethanol yield ($P<0.05$) (Table 3). There were three Alamo x Kanlow combinations, and two Miami x Kanlow combinations. There were two Alamo x Alamo combinations, and one Kanlow x Kanlow combinations (Table 3). The result of three Alamo x Kanlow combinations with high parent heterosis for both predicted leaf and stem ethanol indicated that this particular cross combination could produce higher predicted ethanol yields. In addition, predicted leaf and stem ethanol yields did not differ significantly. Thus, if selecting in the future for ethanol yield an Alamo x Kanlow combination may be desirable.

Phenotypic Correlations

Agronomic Traits

Leaf biomass yield was significantly correlated to all other agronomic traits across both years and locations. It was moderately, positively correlated to total plant biomass yield (0.56), and highly, positively correlated to stem biomass (0.88; Table 4). Stem biomass had significant correlation coefficients between leaf to plant ratio, total biomass yield, and stem biomass across both years and locations ($P<0.05$). As with leaf biomass, stem biomass had a moderate, negative correlation to leaf to plant ratio (-0.65; Table 4). Biologically, in some cases, a plant with a higher stem biomass will have a decreased leaf biomass, effectively lowering the leaf to plant ratio, and increasing the stem to plant ratio. Stem biomass is highly correlated to total yield (0.71), and is generally an indication of the plant's total biomass yield (Table 4).

Leaf to plant ratio had significant correlations with all other agronomic traits across both years and locations ($P<0.05$). Leaf to plant ratio was moderately, negatively correlated to total biomass yield (-0.51; Table 4). Stem biomass yield comprises more of the total yield than leaf biomass yield, thus, if a plant had a larger leaf to plant ratio, there is a greater indication for decreased total biomass yield, resulting in a negative correlation of the two traits. There was a moderate, positive correlation between leaf to plant ratio and predicted plant ethanol yield (Table 4). Leaf to plant ratio was negatively, moderately correlated to leaf biomass (-0.47) and stem biomass (-0.65; Table 4).

Morphological Traits

Leaf biomass per plant was positively correlated to all morphological traits across both years and locations ($P<0.05$). It was moderately correlated to tiller number (0.49), average tiller diameter

(0.59), and average leaf diameter (0.58; Table 5). There was a weak correlation between leaf biomass and height (0.13; Table 5). The leaf diameter in spring may be able to give a moderate indication of total leaf biomass in the fall. Stem biomass was also positively correlated to all morphological traits across both years and locations ($P<0.05$). There was a strong positive correlation between average tiller diameter and stem biomass (0.74; Table 5). Stem biomass was moderately correlated to tiller number in the spring (0.62) as well as leaf diameter (0.65). Stem biomass was more highly correlated with spring height than leaf biomass (Table 5). Based on these correlations, the average tiller diameter taken in the spring will be a good indicator for the stem biomass of the fall-harvested plant. Plants with larger tiller diameter will likely have a greater amount of stem biomass yield. With weaker correlations to height for both stem and leaf biomass; this would not be an adequate indication of the plant's performance in the fall for these traits. Total biomass yield was highly correlated to tiller number (0.86), indicating that tiller number in the spring gave evidence to the total biomass yield in the fall. This is congruent with the research findings of Boe and Beck (2008), which stated that biomass, was highly correlated to tiller number. In addition, total biomass yield was also highly correlated to tiller diameter (0.75), as predicted by Das et al. (2004). This provided further evidence that tiller number and diameter are helpful indicators for biomass production. There was a moderate correlation between leaf diameter and total biomass yield (0.65). Height was moderately correlated to total biomass yield (0.36; Table 5).

CHAPTER V

Summary

Clonal replicates of F_1 plants of 20 crosses resulting from a 5x5 parent diallel were evaluated for yields and morphological traits at two locations (Knoxville and Crossville, TN) for two years (2012 and 2013). The results indicated that the two locations gave similar results and that either of the locations would have been sufficient to evaluate the crosses; both were not necessary, except to guard against catastrophic losses. There were significant differences between the two years, as the plants during the second year increased in productivity as they approached their maximum productivity that occurs in year 3-4 (Garland, 2008). There were significant differences among plants within crosses as well as among crosses for most of the traits evaluated.

As expected, there was an increase in leaf biomass yield and stem biomass yield at both locations the second year as plants continued to develop morphologically. Switchgrass does not reach its full maturity until the third or fourth year. However, nearly 70% of biomass is produced by the second year, thus it is difficult to adequately compare the first and second year of data (Garland, 2008).The second year is more indicative of how plants are likely to perform when they reach their full capacity. However, the cross rankings were similar across years so the first year results were reasonably reliable in regard to how crosses were going to perform in rank.

In terms of leaf biomass yield per plant, overall population means increased from 2012 to 2013, as expected due to increasing maturity. The overall population mean for leaf biomass increased approximately 27 g plant^{-1} from 2012 to 2013. The F_1 progeny of K2xM and T1 x M were the top performing crosses for leaf biomass yield, averaging 71.9 g plant^{-1}. In addition, both of the top performing crosses for leaf biomass contained the M parental line, which had the largest

GCA for this trait. If selection were to continue for the leaf biomass yield trait, selecting and advancing progeny of T1xM would be advised. In all cases, the F_1 progeny of K2xK3 was the lowest performing cross for leaf biomass yield and also had the lowest SCA for this trait. Leaf biomass had very low narrow sense heritability and much larger broad sense heritability. These results indicate there was very little additive variance among F_1's of these crosses, and a larger proportion of non-additive variance. The Kanlow parental lines were less genetically diverse than Alamo parental lines, thus the cross of two Kanlow lines resulted in less yield and lower combining abilities overall.

Similar to leaf biomass yield, stem biomass yield values increased from 2012 to 2013, as expected. The increase in stem biomass yield was larger than the increase in leaf biomass yield. As the plants age and progress toward maximum production potential, the stems increased in number and diameter. Correlations indicated that a plant with larger tiller diameter and tiller number would produce larger plant biomass. The F_1 progeny of A1xK2 was the highest performing plant for stem biomass yield. The lowest performing cross across both years and locations was K3xM, which had approximately 50 g plant^{-1} less stem biomass yield than the highest performing cross A1xK2. The cross K3xM contains the Miami parent, which is a leafy and robust line that has a high leaf:plant biomass ratio. The reciprocal cross MxK3 had the highest leaf to plant ratio (0.75), containing only 25% stem biomass. There were no significant reciprocal effects between K3xM and MxK3, indicating that both of these crosses had higher proportions of leaves compared to stems. This parental combination is not ideal for selection of stem biomass yield but might be desirable for producing forage lines. In addition, there is little overlap between crosses when trying to select for a plant containing both high leaf biomass yield and high stem biomass yield. In this case, it is best to consider the total biomass yield. There

were reciprocal effects observed between cross MxT1 and T1xM across both years and locations. This is supported by T1xM having the largest SCA value for stem biomass yield and its reciprocal MxT1 having the lowest SCA value.

The leaf to plant ratio decreased from 2012 to 2013. As the plants aged and progressed toward their maximum potential, both leaf and stem biomass increased. The F_1 progeny of MxK3 had the largest leaf to plant ratio across both years and locations. However, across both years and locations this cross had the lowest total biomass yield, again indicating that the F_1 progeny had a higher proportion of leaves. In the vast majority of cases, comparing the maximum and minimum means to find an ideal cross does not seem to yield helpful results, as these crosses generally have conflicting results for biomass traits such as leaf biomass yield, stem biomass yield, and leaf to plant ratio. On the other hand, the crosses studied herein represent an array of options available to switchgrass breeders. For example, some crosses would be better suited for selecting high biomass lines (e.g. T1xM and A1xK2), other better for forage lines (e.g. MxK3), and others for integrated forage/biomass lines.

The predicted ethanol yields from leaves versus stems did not differ when compared across yeas and locations. Thus for these crosses it does not seem advantageous to select for more leaves than stems or vice versa. This infers that it would have been possible to select for ethanol yield in the first year (Sykes, Ph.D. Dissertation, 2014). Due to this, it is best to examine the total predicted plant ethanol values coupled with total biomass yield for future breeding efforts.

There was significant high parent heterosis for leaf biomass yield. The cross with the largest high parent heterosis and SCA across both years and locations was T1xM. Furthermore this cross also had the largest high parent heterosis value and highest SCA values for stem biomass yield and

overall plant biomass but it was not the highest performing cross in terms of stem biomass yield. The highest performing cross for stem biomass yield in combined years and locations was A1xK2, which did have significant high parent heterosis as well, indicating this cross could work well in hybrid combination. There is evidence of a small amount high parent heterosis in the cross T1xK2 for the trait leaf to plant ratio; however, other traits for this cross were not significant.

Due to the large amount of non-additive variation of the traits studied, and specific examples of high parent heterosis, it appears that F_1 hybrid development warrants consideration. Leaf biomass yield, stem biomass yield, leaf to plant ratio, predicted leaf ethanol yield and predicted stem ethanol yield all displayed occurrences of higher dominance values compared to additive values. Hybrid breeding methods are better suited for exploiting this type of genetic variance, especially for leaf and stem biomass. Both of these traits exhibited much higher dominance values than additive. Predicted leaf and stem ethanol yields did display higher dominance variance, although these values were closer in comparison to the biomass traits. As stated previously, leaf and stem ethanol values did not differ significantly ($P>0.05$), and selection would likely not be focused on these traits but on the total shoot values. Thus, hybrid selection should be focused towards biomass yield. The high parent heterosis associated with the biomass traits also indicates hybrid selection, which exploits the use of high parent heterosis for future selection, or hybrid vigor. The crosses K2xM and K2xT1 both exhibited significant ($P<0.05$) high parent heterosis for leaf and stem biomass yield. Low h^2 values but high H^2 values, as displayed here also indicate hybrid selection as being ideal.

Based on results of this study, breeder's efforts for future selection for biofuel type varieties should be focused on overall biomass yield and overall predicted ethanol yield. The highest

performing crosses for leaf biomass yield and stem biomass yield did not equate to the highest overall biomass yield. A larger stem biomass (kg DM plant^{-1}) does not equate to a larger stem ethanol or leaf ethanol value (mg g^{-1} DM). Leaf and stem biomass yield are both moderately correlated to tiller number, tiller diameter, and leaf diameter. Knowing the values for tiller number, tiller diameter, and leaf diameter would give some indication of leaf and stem biomass yield, as well as overall biomass yield. Breeding efforts would be better focused on examining the overall yield and balancing traits such as tiller number and plant height.

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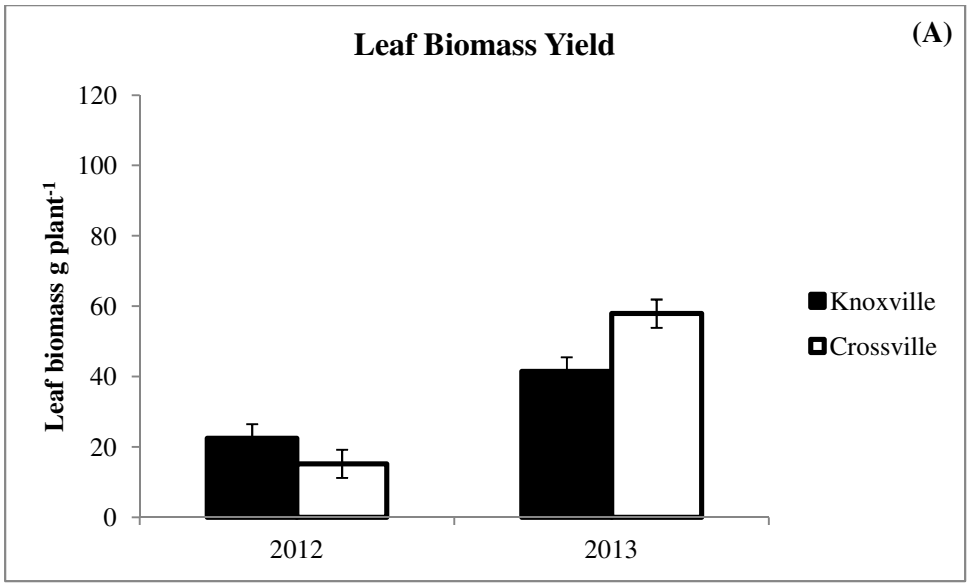
Appendices



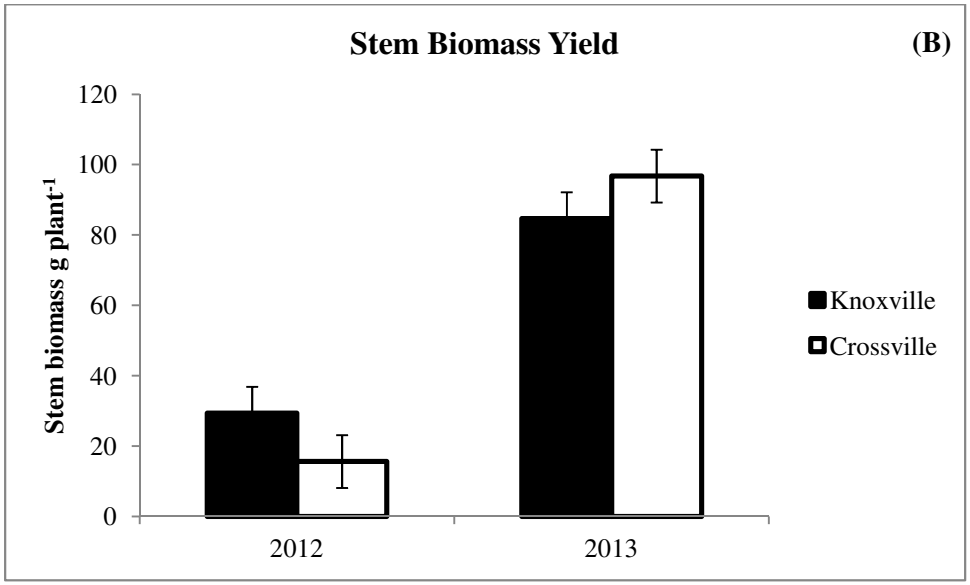


Fig.1. Average leaf (A) and stem (B) biomass yield averaged across 20 switchgrass crosses for 2012 and 2013 at Knoxville and Crossville, TN. Vertical lines are standard error of means.

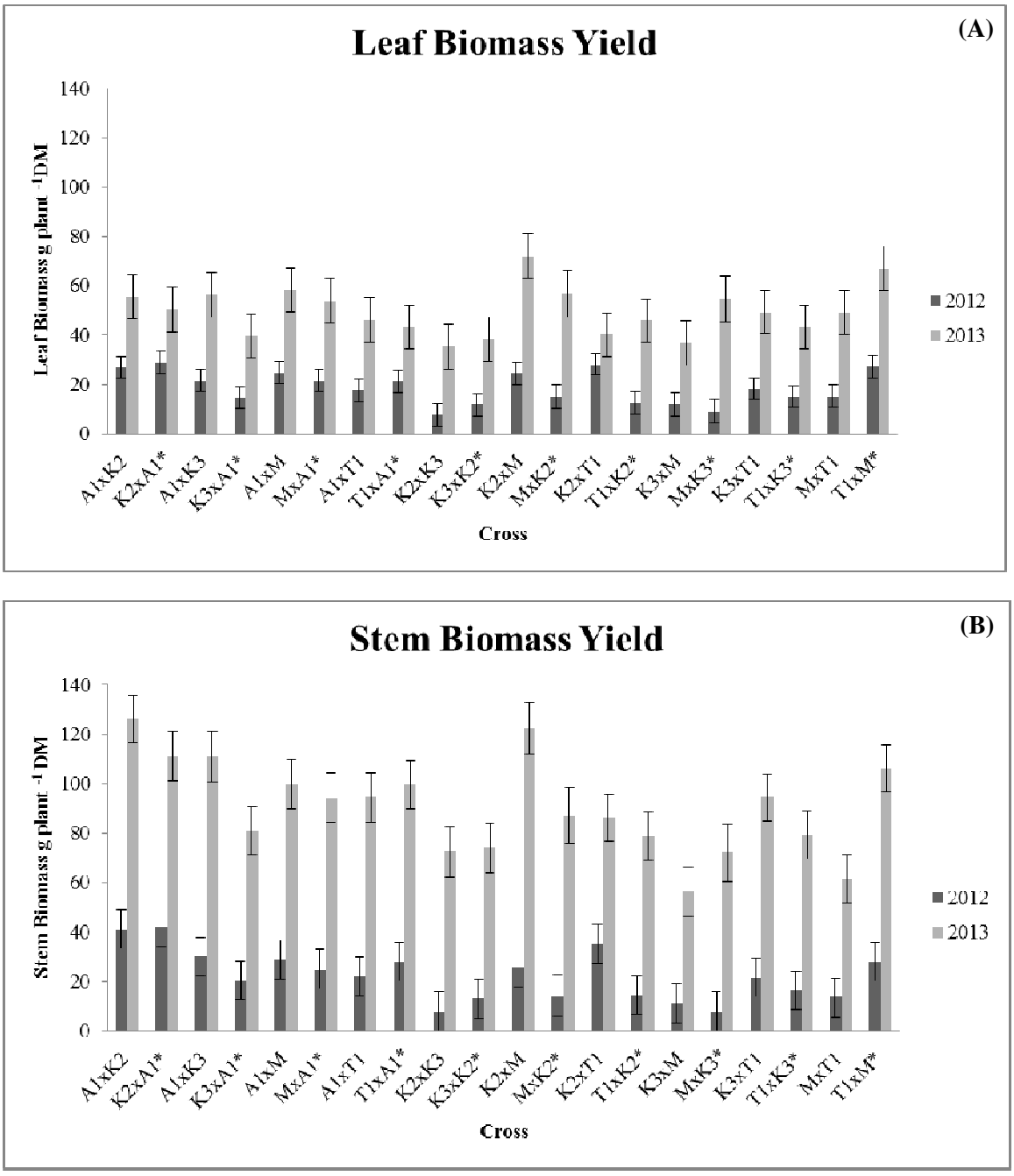


Fig.2. Bar graphs for leaf (A) and stem (B) biomass yield for 20 switchgrass crosses (including 10 reciprocals*) averaged across Knoxville and Crossville, TN locations for 2012 and 2013.Vertical lines are standard errors of means. Parental lines A1 and T1 were derived from cv. Alamo; K2 and K3 were derived from cv. Kanlow; and M was derived from cv. Miami.

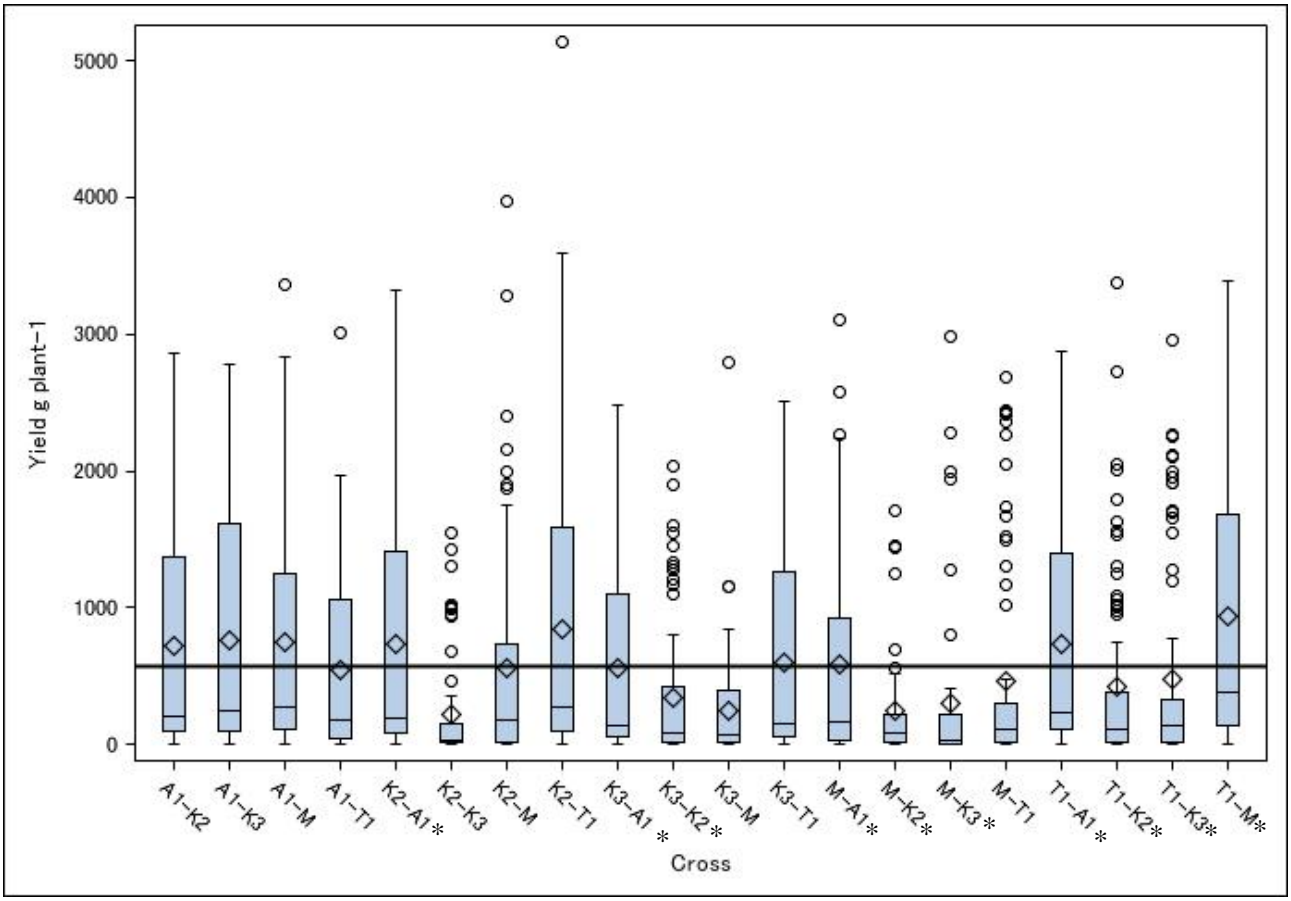


Fig.3. Box plot of plant biomass yield of 20 switchgrass crosses (including ten reciprocals*) across Knoxville and Crossville, TN locations and two years (2012 and 2013). The solid horizontal line indicates the overall population mean. Individual cross means are represented by diamonds. Median of each cross is represented by line inside box, and circles represent cross outliers. Parental lines A1 and T1 were derived from cv. Alamo; K2 and K3 were derived from cv. Kanlow; and M was derived from cv. Miami.

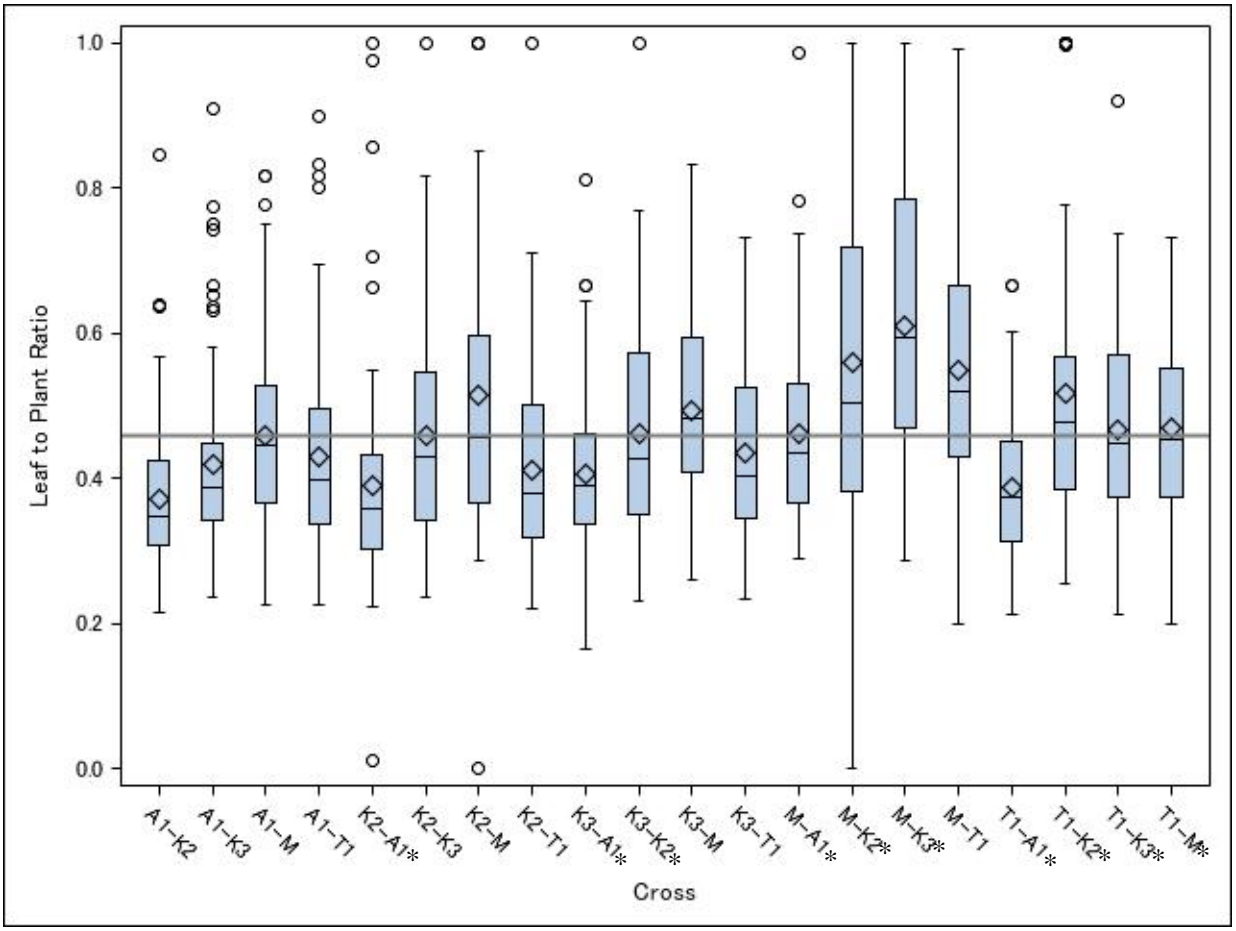
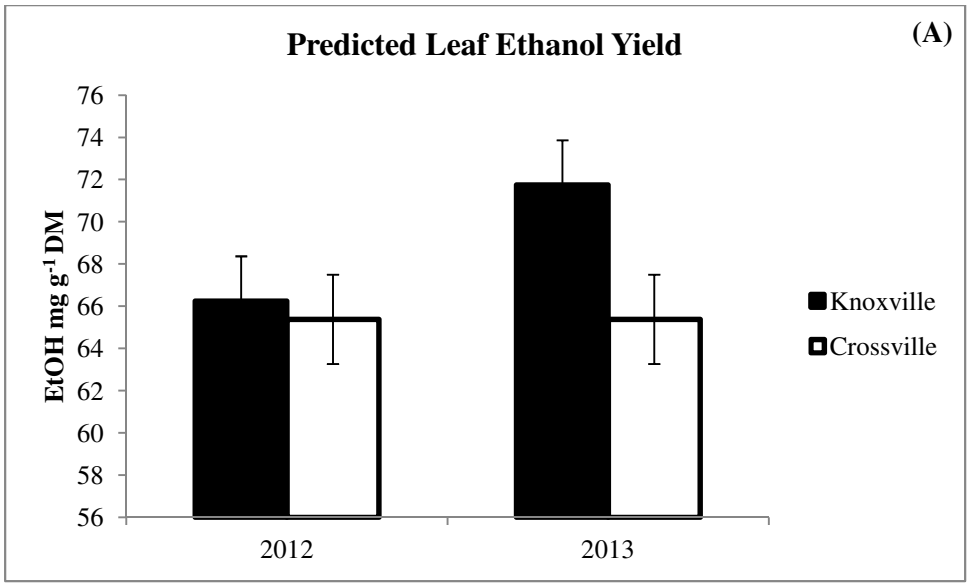


Fig.4. Box plot of leaf:plant biomass ratio averaged across Knoxville and Crossville, TN in 2012 and 2013 for 20 switchgrass crosses (including ten reciprocals*). The solid horizontal line indicates the overall population mean. Individual cross means are represented by diamonds. Median of each cross is represented by line inside box, and circles represent cross outliers. Parental lines A1 and T1 were derived from cv. Alamo; K2 and K3 were derived from cv. Kanlow; and M was derived from cv. Miami.



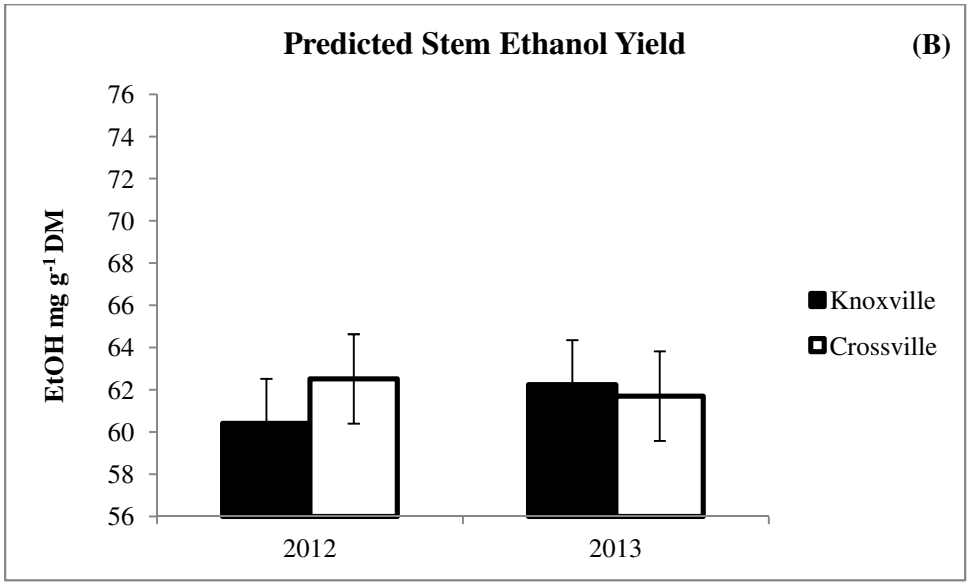
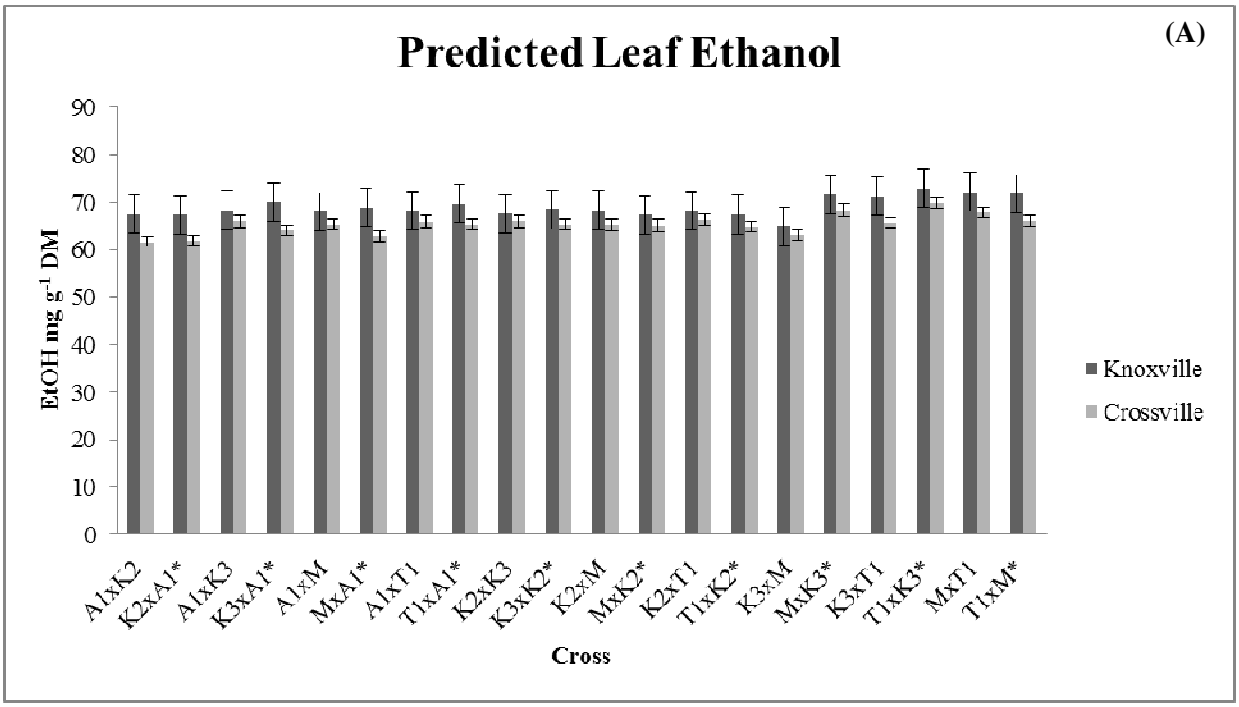


Fig.5. Averaged predicted leaf (A) and stem (B) ethanol yield averaged across 20 switchgrass crosses for 2012 and 2013 at Knoxville and Crossville, TN. Vertical lines are standard error of means.



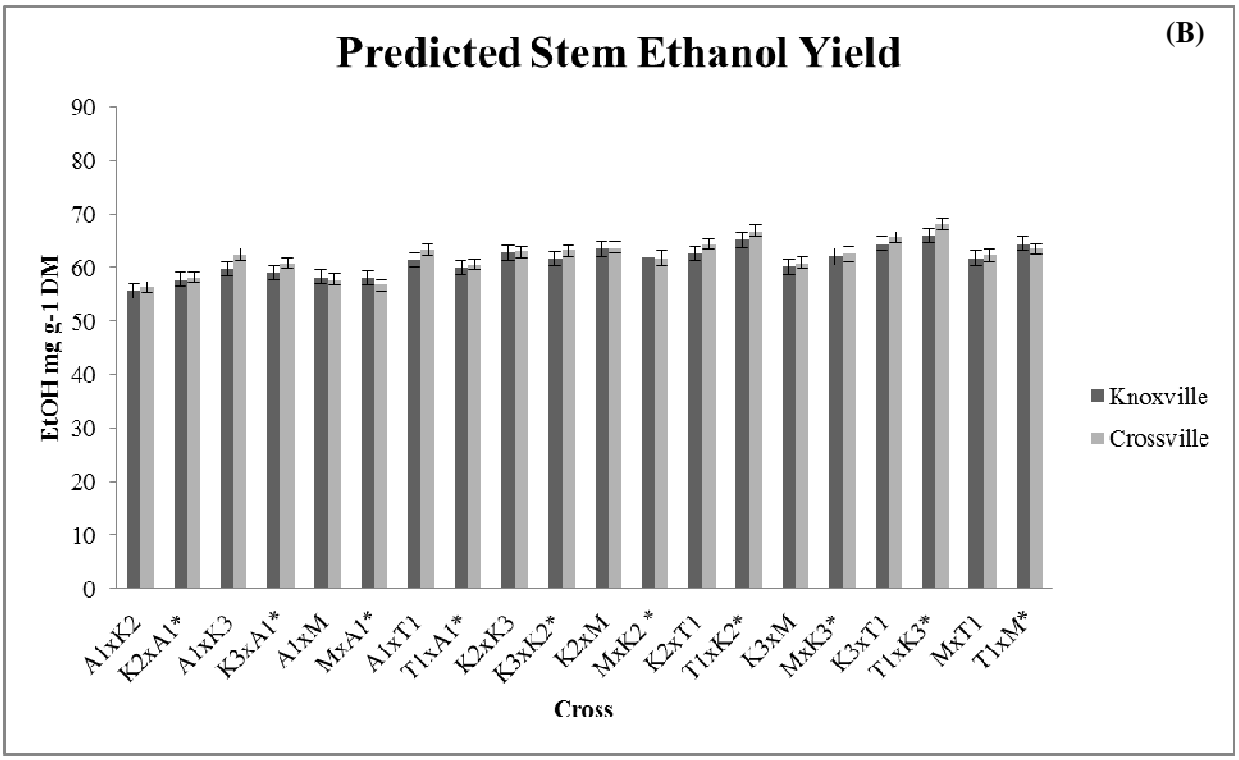


Fig.6. Average predicted leaf (A) and stem (B) ethanol yield averaged across 20 switchgrass crosses (including ten reciprocals*) for Knoxville and Crossville, TN (A) across 2012 and 2013. Vertical lines are standard error of means. Parental lines A1 and T1 were derived from cv. Alamo; K2 and K3 were derived from cv. Kanlow; and M was derived from cv. Miami.

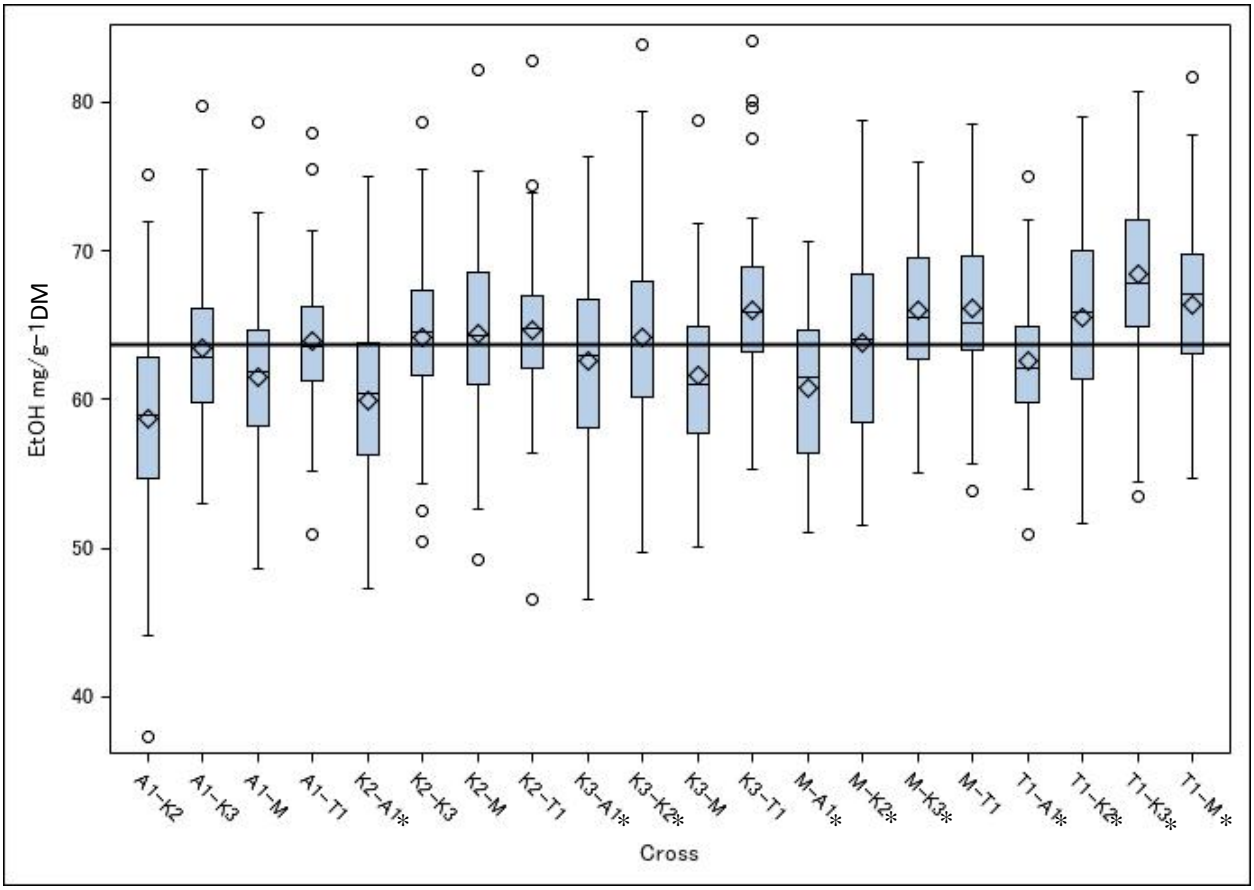


Fig.7. Box plot for total predicted plant ethanol yield of 20 switchgrass crosses (including ten reciprocals*) across Knoxville and Crossville, TN locations and two years (2012 and 2013). The dark line indicates the overall population mean. Individual cross means are represented by diamonds. Median of each cross is represented by line inside box, and circles represent cross outliers. Parental lines A1 and T1 were derived from cv. Alamo; K2 and K3 were derived from cv. Kanlow; and M was derived from cv. Miami.

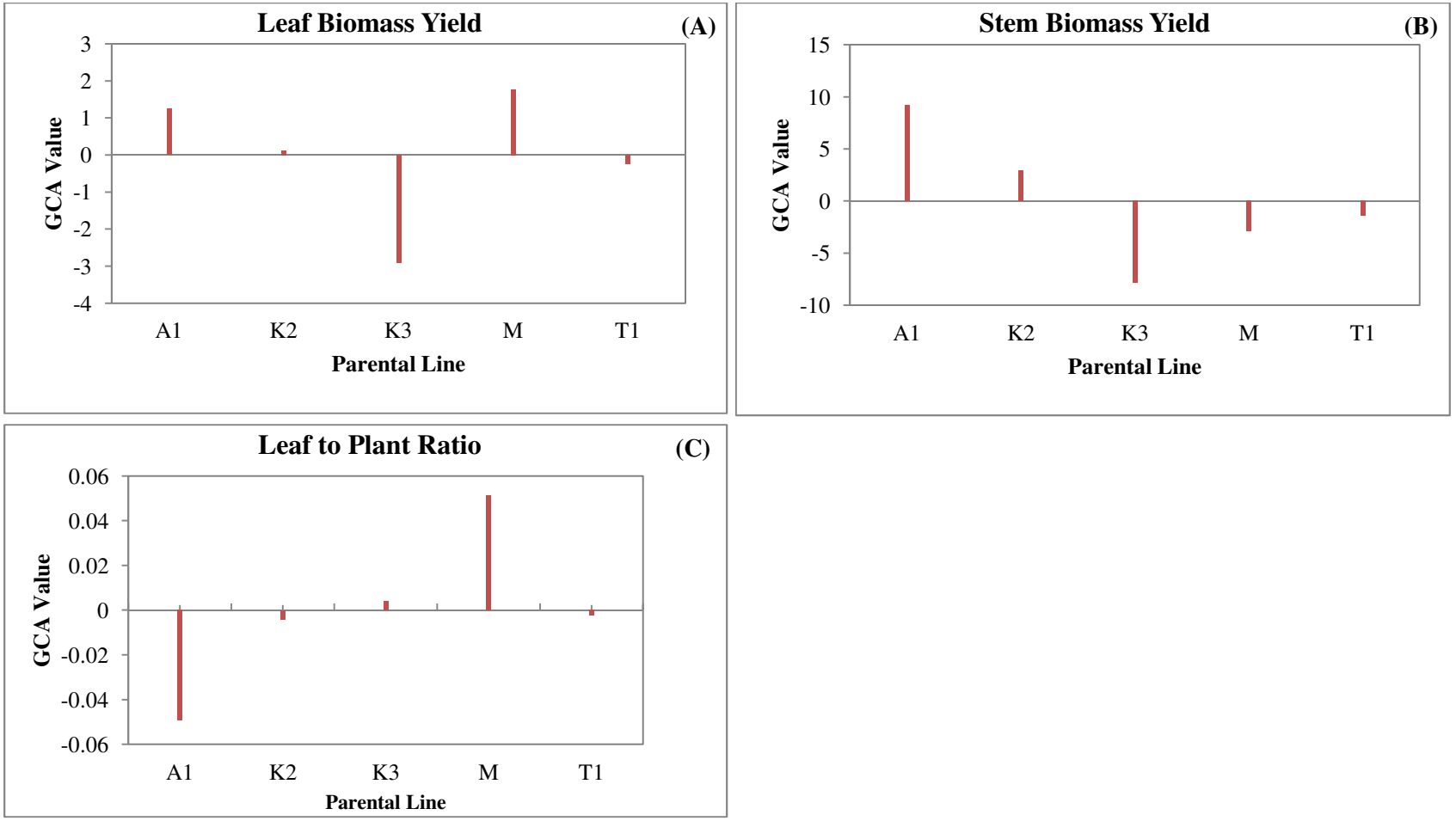


Fig.8. Plots for general combining ability (GCA) for leaf biomass yield (A), stem biomass yield (B), and leaf:plant ratio (C) for five switchgrass parental lines for 20 crosses across Knoxville and Crossville, TN and 2012 and 2013. Parental lines A1 and T1 were derived from cv. Alamo; K2 and K3 were derived from cv. Kanlow; and M was derived from cv. Miami.

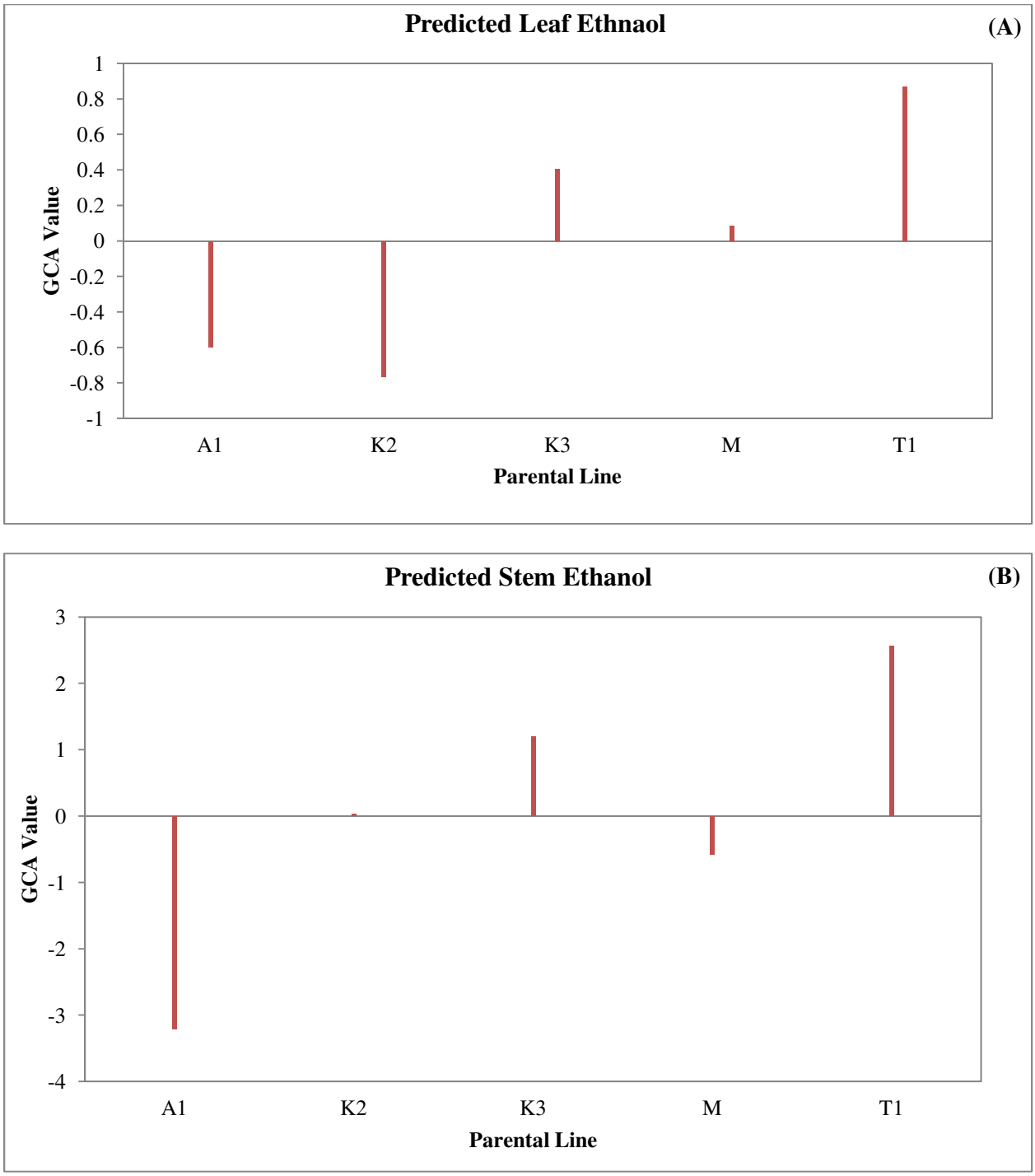


Fig.9. Plots for general combining ability for predicted leaf (A) and stem (B) ethanol yield of five switchgrass parental lines for 20 crosses (including ten reciprocals) across Knoxville and Crossville, TN and 2012 and 2013. Parental lines A1 and T1 were derived from cv. Alamo; K2 and K3 were derived from cv. Kanlow; and M was derived from cv. Miami.

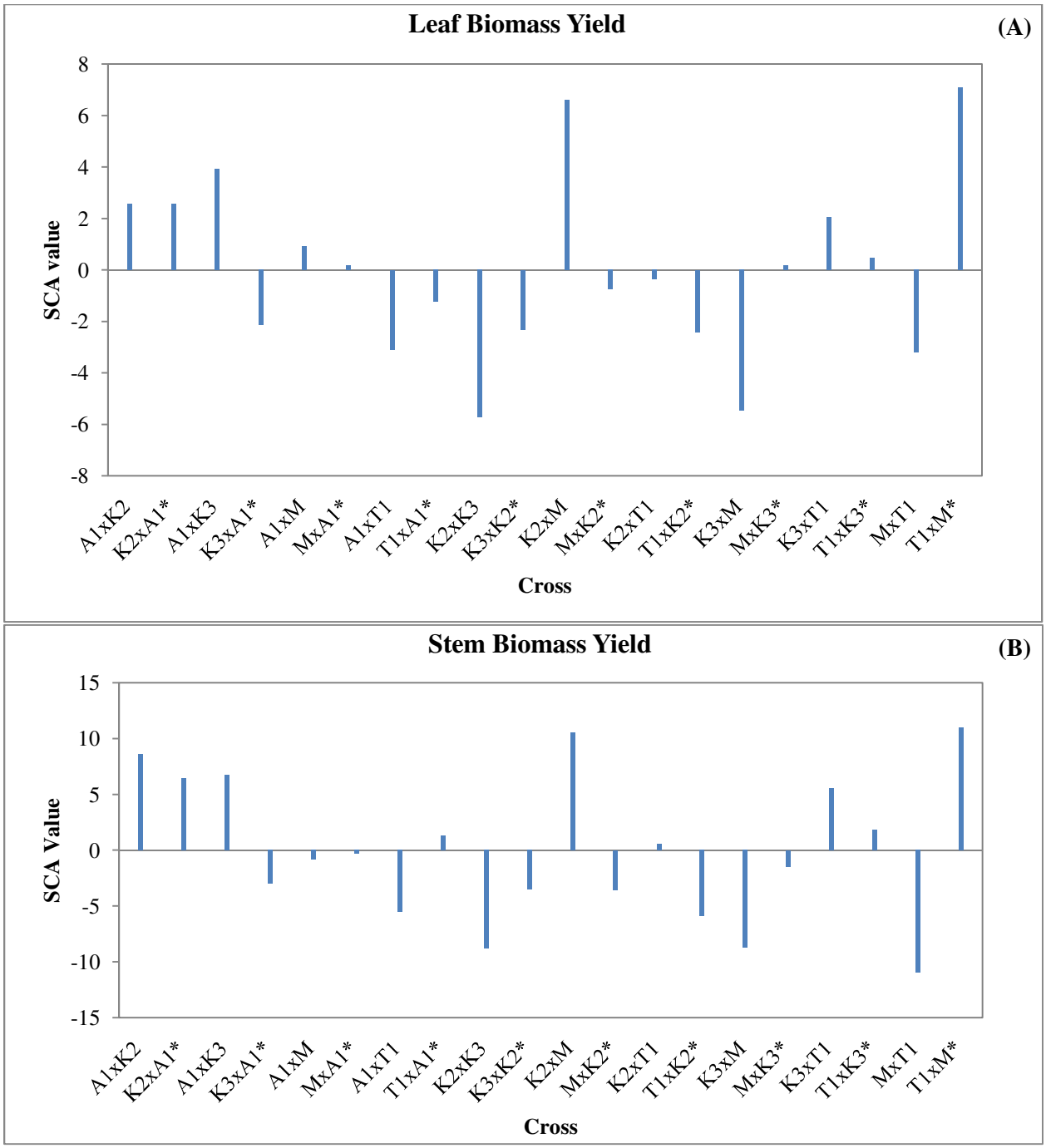


Fig.10. Plots for specific combining ability (SCA) for leaf (A) and stem (B) biomass yield for 20 switchgrass crosses (including ten reciprocals*) across Knoxville and Crossville, TN in 2012 and 2013. Parental lines A1 and T1 were derived from cv. Alamo; K2 and K3 were derived from cv. Kanlow; and M was derived from cv. Miami.

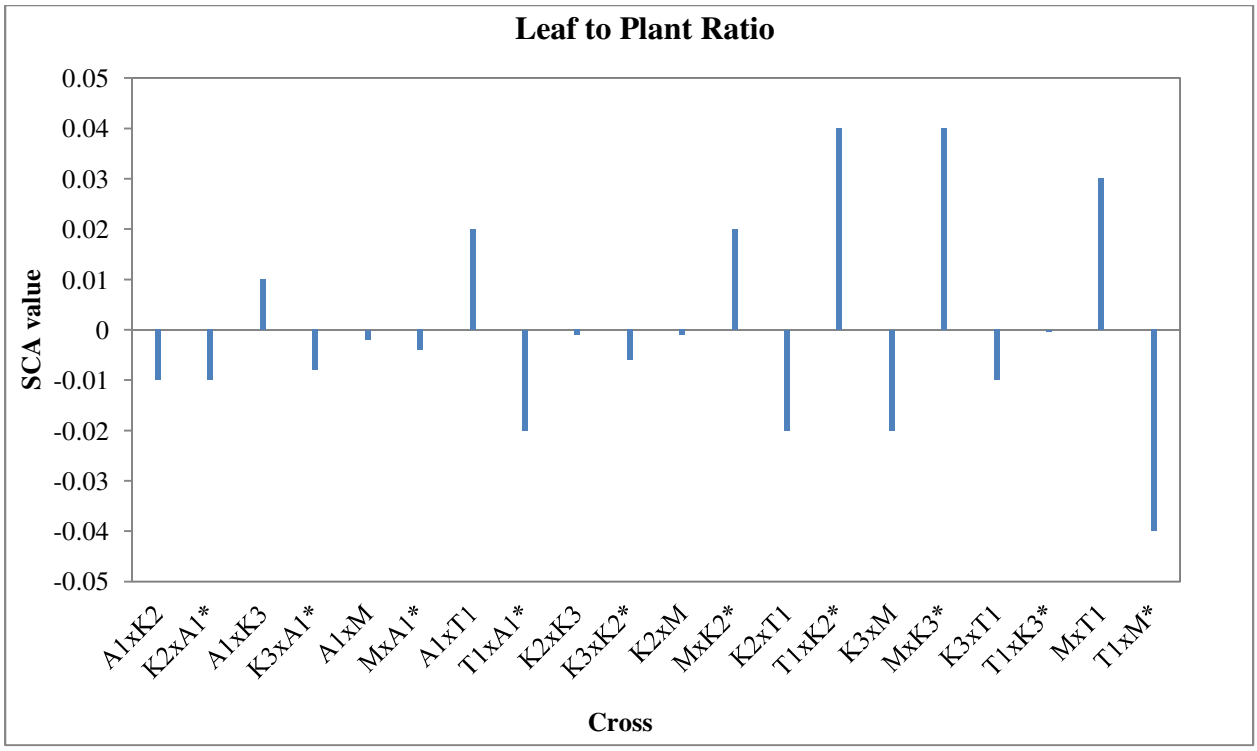


Fig.11. Plots for specific combining ability (SCA) for leaf:plant ratio for 20 switchgrass crosses (including ten reciprocals*) across Knoxville and Crossville, TN in 2012 and 2013. Parental lines A1 and T1 were derived from cv. Alamo; K2 and K3 were derived from cv. Kanlow; and M was derived from cv. Miami.

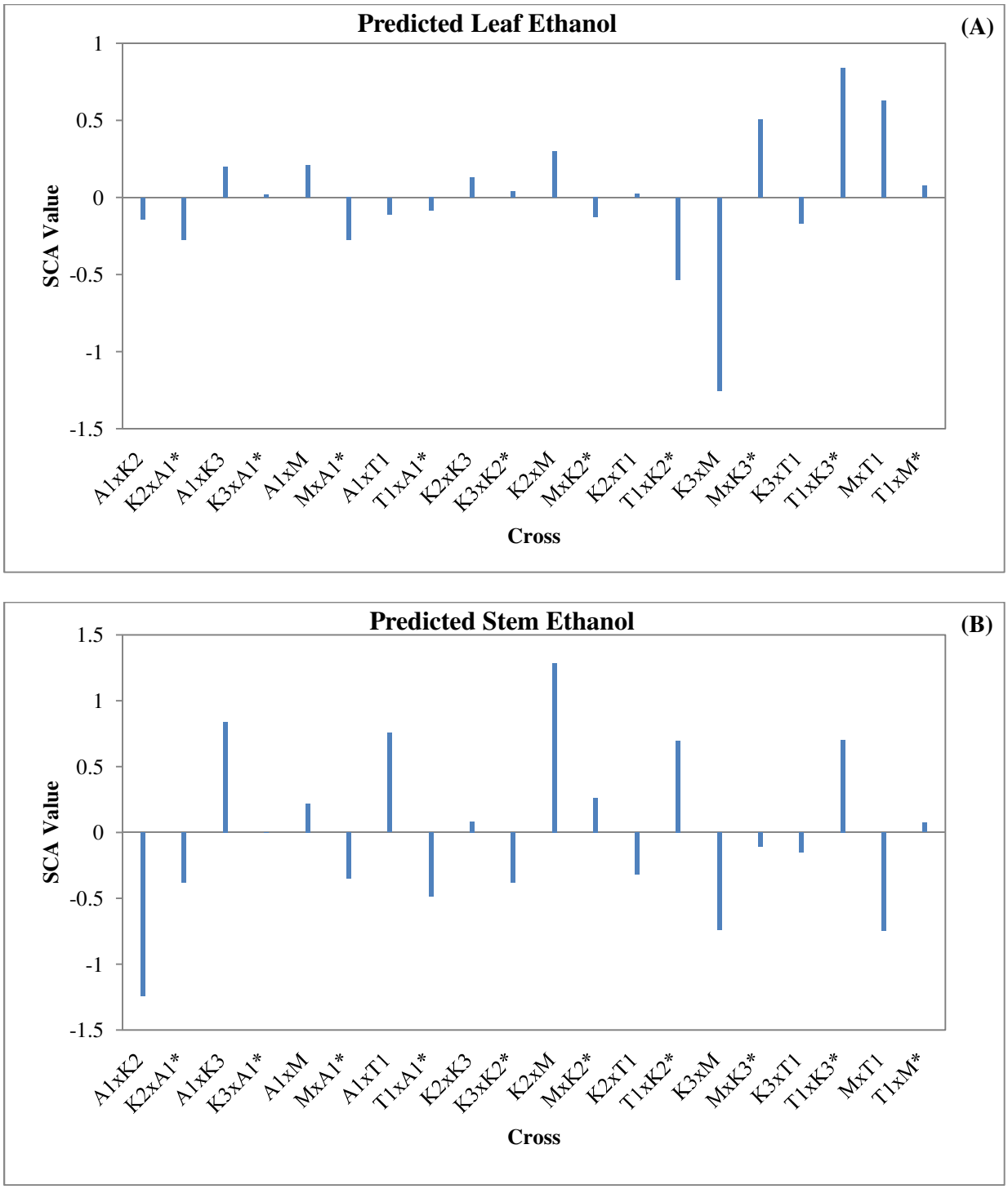


Fig.12. Plots for specific combining ability (SCA) for predicted leaf (A) and stem (B) ethanol yield for 20 switchgrass crosses (including ten reciprocals*) across Knoxville and Crossville, TN in 2012 and 2013. Parental lines A1 and T1 were derived from cv. Alamo; K2 and K3 were derived from cv. Kanlow; and M was derived from cv. Miami.

Table.1. Additive variance, dominance variance, phenotypic variance, narrow sense heritability (h2), and broad sense heritability (H2) for four yield components across 20 switchgrass crosses (including 10 reciprocals) located at Knoxville and Crossville, TN in 2012 and 2013

	Leaf biomass (g plant $^{-1}$)	**Stem biomass (g plant $^{-1}$)**	**Predicted Leaf Ethanol Yield (mg/g^{-1} DM)**	**Predicted Stem Ethanol Yield (mg/g $^{-1}$ DM)**
Parameter				
σ^2_A	14.93	172.71	3.03	19.69
σ^2_D	146.70	342.47	3.96	30.56
σ^2_P	503.05	1629.83	42.95	83.84
h^2 (Narrow Sense)	0.03	0.11	0.07	0.23
H^2 (Broad sense)	0.32	0.32	0.16	0.6

Table.2. High parent heterosis values for leaf and stem biomass and leaf to plant ratio across 20 switchgrass crosses and five parental lines at Knoxville and Crossville, TN in 2012 and 2013. Mean values for each trait are listed as well as the high parent from each cross. High parent heterosis values are present. Parental lines A1 and T1 were derived from cv. Alamo; K2 and K3 were derived from cv. Kanlow; and M was derived from cv. Miami.

*Significant at the 0.05 probability level

Cross	Leaf Biomass Yield	High Parent	High Parent Heterosis	Stem Biomass Yield	High Parent2	High Parent Heterosis2	Leaf to Plant Ratio	High Parent3	High Parent Heterosis3
A1 Parent	25.99			55.06			0.44		
A1xK2	41.83	A1	15.84	84.51	A1	29.451*	0.37	A1	-0.06
A1xK3	37.76	A1	11.77	67.64	A1	12.58	0.42	A1	-0.01
A1xM	40.09	M	10.63	61.16	A1	6.10	0.46	M	-0.17
A1xT1	30.40	A1	4.41	54.87	A1	-0.19	0.43	A1	0.00
K2 Parent	20.78			40.08			0.43		
K2xA1	38.94	A1	12.95	74.27	A1	19.21	0.39	A1	-0.05
K2xK3	20.75	K2	-0.04	37.56	K2	-2.52	0.46	K2	0.02
K2xM	46.42	M	16.9612*	70.27	K2	30.1927*	0.51	M	-0.12
K2xT1	34.45	K2	13.6627*	60.76	K2	20.6875*	0.41	T1	-0.02
K3 Parent	19.73			35.01			0.41		
K3xA1	25.95	A1	-0.03	48.41	A1	-6.65	0.41	A1	-0.03
K3xK2	24.71	K2	3.93	42.61	K2	2.54	0.46	K2	0.03
K3xM	23.84	M	-5.62	32.86	K3	-2.15	0.49	M	-0.14
K3xT1	33.31	K3	13.58	56.74	K3	21.72	0.44	T1	0.00
M Parent	29.03			28.44			0.63		
MxA1	37.67	M	8.21	59.20	A1	4.14	0.46	M	-0.17
MxK2	33.92	M	4.461*	47.40	K2	7.32	0.56	M	-0.07
MxK3	28.03	M	-1.43	34.93	K3	-0.08	0.61	M	-0.02
MxT1	31.94	M	2.48	36.93	T1	2.45	0.55	M	-0.08
T1 Parent	19.02			34.48			0.43		
T1xA1	31.79	A1	5.80	62.06	A1	7.01	0.39	A1	-0.05
T1xK2	28.45	K2	7.67	45.11	K2	5.03	0.52	K2	0.082*
T1xK3	28.87	K3	9.14	47.07	K3	12.05	0.47	T1	0.03
T1xM	46.70	M	17.2372*	66.37	T1	31.8888*	0.47	M	-0.16

Table.3. High parent heterosis values for predicted leaf and stem ethanol yield 20 switchgrass crosses and five parental lines at Knoxville and Crossville, TN in 2012 and 2013. Mean values for each trait are listed as well as the high parent from each cross. High parent heterosis values are present. Parental lines A1 and T1 were derived from cv. Alamo; K2 and K3 were derived from cv. Kanlow; and M was derived from cv. Miami.

*Significant at the 0.05 probability level

Cross	Predicted Leaf Ethanol	High Parent	High Parent Heterosis	Predicted Stem Ethanol	High Parent	High Parent Heterosis
A1 Parent	65.75	N/A	N/A	56.6	N/A	N/A
A1xK2	65.53	A1	-0.22	55.6	K2	-3.75
A1xK3	67.12	K3	0.90	61.0	K3	-1.79
A1xM	66.71	M	0.05	58.0	M	0.83
A1xT1	66.98	T1	-2.19	62.3	T1	-2.11
K2 Parent	61.78	N/A	N/A	59.3	N/A	N/A
K2xA1	64.58	A1	-1.17	57.9	K2	-1.38
K2xK3	66.59	K3	0.37*	62.8	K3	0.01*
K2xM	66.70	M	0.04*	63.5	K2	4.13
K2xT1	67.10	T1	-2.07	63.5	T1	-0.84
K3 Parent	66.22	N/A	N/A	62.8	N/A	N/A
K3xA1	67.13	K3	0.91	59.9	K3	-2.93
K3xK2	66.86	K3	0.64	62.4	K3	-0.41
K3xM	64.13	M	-2.53	60.6	K3	-2.27
K3xT1	68.36	T1	-0.81	65.0	T1	0.65
M Parent	66.66	N/A	N/A	57.2	N/A	N/A
MxA1	65.82	M	-0.84	57.4	M	0.22
MxK2	65.96	M	-0.70	61.9	K2	2.57
MxK3	69.54	M	2.88	62.3	K3	-0.53
MxT1	70.07	T1	0.91	62.0	T1	-2.38
T1 Parent	69.17	N/A	N/A	64.4	N/A	N/A
T1xA1	67.39	T1	-1.78	60.4	T1	-4.03
T1xK2	66.14	T1	-3.02	66.0	T1	1.67*
T1xK3	71.39	T1	2.22*	67.1	T1	2.72*
T1xM	68.98	T1	-0.19	64.1	T1	-0.27

Table.4. Phenotypic correlation coefficients among yield components of 20 switchgrass crosses (including ten reciprocals) located at Knoxville and Crossville, TN in 2012 and 2013.

Yield Component	Leaf to Plant Ratio	Leaf biomass (g plant^{-1})	Stem biomass (g plant^{-1})	Total Biomass Yield (g plant^{-1})
Leaf biomass	-0.47*			
Stem biomass	-0.65*	0.88*		
Total Biomass Yield	-0.51*	0.56*	0.71*	
Predicted Plant Ethanol Yield	0.32*	-0.19*	-0.22*	-0.08*

*Significant at the 0.05 probability level

Table.5. Phenotypic correlation coefficients among yield components and morphological traits of 20 switchgrass crosses (including ten reciprocals) located at Knoxville and Crossville, TN in 2012 and 2013.

	Leaf to Plant Ratio	**Leaf biomass (g plant^{-1})**	**Stem biomass (g plant^{-1})**	**Total Biomass Yield (g plant^{-1})**
Tiller Number	-0.48*	0.49*	0.62*	0.86*
Tiller Diameter (mm)	-0.60*	0.59*	0.74*	0.61*
Leaf Diameter (mm)	-0.52*	0.58*	0.65*	0.56*
Height in Spring (cm)	-0.33*	0.13*	0.30*	0.36*

*Significant at the 0.05 probability level

Appendix A

Table.1A. Cross combinations of F_1 progeny including female, male, and reciprocal cross as well as parental lines derived from Alamo, Kanlow and Miami Varieties.

Cross Combination	**Female**	**Male**	**Reciprocal Cross**
A1xK2	A1	K2	K2xA1
A1xK3	A1	K3	K3xA1
A1xM	A1	M	MxA1
A1xT1	A1	T1	T1xA1
K2xA1	K2	A1	A1xK2
K2xK3	K2	K3	K3xK2
K2xM	K2	M	MxK2
K2xT1	K2	T1	T1xK2
K3xA1	K3	A1	A1xK3
K3xK2	K3	K2	K2xK3
K3xM	K3	M	MxK3
K3xT1	K3	T1	T1xK3
MxA1	M	A1	A1xM
MxK2	M	K2	K2xM
MxK3	M	K3	K3xM
MxT1	M	T1	T1xM
T1xA1	T1	A1	A1xT1
T1xK2	T1	K2	K2xT1
T1xK3	T1	K3	K3xT1
T1xM	T1	M	MxT1

Parental Line	Parental Derivative
A1	Alamo
K2	Kanlow
K3	Kanlow
M	Miami PI
T1	Alamo

Table.1B. Phenotypic Trait Measurements

Traits	***Measurement***
Tiller Number	Count
Plant Height	cm
Tiller Diameter	mm
Leaf Diameter	mm
Total Biomass Yield	Whole plant harvest with a representaive sample take to account for mositure (g plant^{-1})
Leaf Biomass Yield	Sample of approximately ten tillers sampled from whole plant and futher separeated manually from stem biomass (g plant^{-1})
Stem Biomass Yield	Sample of approximately ten tillers sampled from whole plant and further separated manually from leaf biomass (g plant^{-1})
Total Predicted Plant Ethanol Yield	Leaf and stem biomass samples are ground and analyzed separately using NIRS analysis. Ethanol yield calculated using adjusted ethanol equation accounting for leaf and stem component of whole plant (mg g^{-1} DM)
Predicted Leaf Ethanol Yield	Ground sample analyzed using NIRS analysis. Ethanol yield calculated using adjusted ethanol equation (mg g^{-1} DM)
Predicted Stem Ethanol Yield	Ground sample analyzed using NIRS analysis. Ethanol yield calculated using adjusted ethanol equation (mg g^{-1} DM)

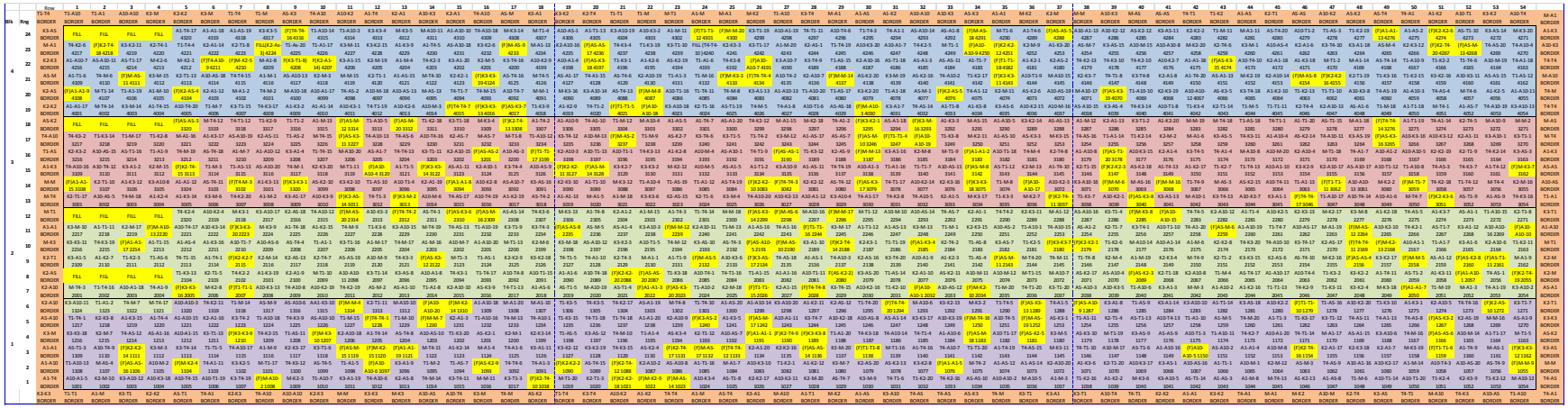


Fig.1A. Map of F_1 population located at East Tennessee Research and Education Center, Holston Unit, Knoxville, TN.

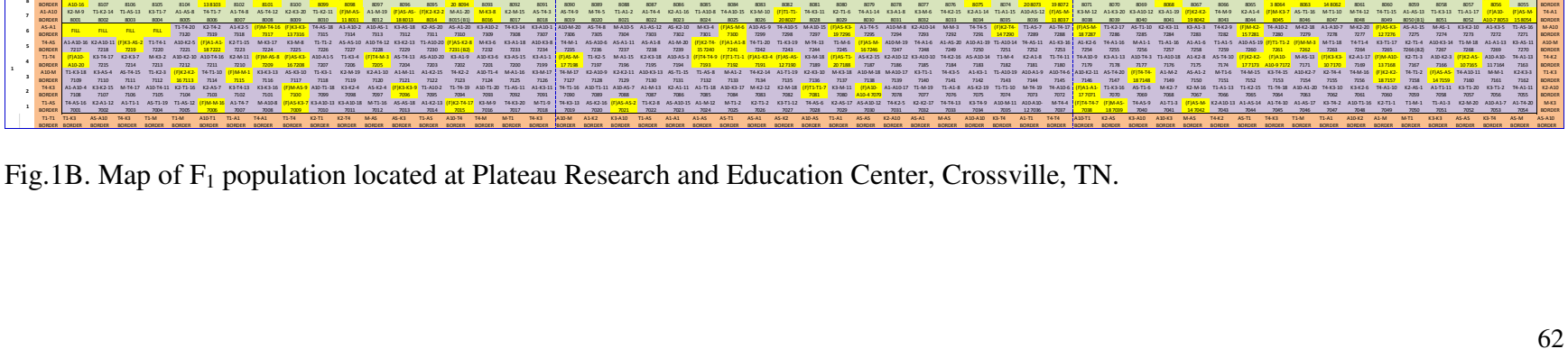


Fig.1B. Map of F_1 population located at Plateau Research and Education Center, Crossville, TN.

Vita

Alexandria Christina DeSantis was born on January 27, 1990 in Canton, Ohio. She graduated from Independence High School in Thompson's Station, Tennessee in 2008. She earned her bachelor's degree in Plant Sciences, concentrating in horticulture production and science, from the University of Tennessee-Knoxville in August 2012. After working with various professors in the Plant Sciences department, including Dr. Fred Allen in the Agronomic State Variety Trials, she decided to continue her education as a graduate research assistant in the Plant Sciences Department at the University of Tennessee-Knoxville in 2013. She is currently pursuing her Master of Science Degree in Plant Sciences with a concentration in Plant Breeding. She will graduate in spring 2015 and begin working with Seminis Seed as a research associate in their lettuce breeding program.