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Genetic Variation for Biomass Yield and Predicted Genetic Gain in Lowland Switchgrass "Kanlow"

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Abstract: Switchgrass (*Panicum virgatum* L.) is a warm-season, perennial grass valued as a promising candidate species for bioenergy feedstock production. Biomass yield is the most important trait for any bioenergy feedstock. This study was focused on understanding the genetics underlying biomass yield and feedstock quality traits in a "Kanlow" population. The objectives of this study were to (i) assess genetic variation (ii) estimate the narrow sense heritability, and (iii) predict genetic gain per cycle of selection for biomass yield and the components of lignocelluloses. Fifty-four Kanlow half-sib (KHS) families along with Kanlow check were planted in a randomized complete block design with three replications at two locations in Tennessee: Knoxville and Crossville. The data were recorded for two consecutive years: 2013 and 2014. The result showed a significant genetic variation for biomass yield ($p < 0.05$), hemicellulose concentration ($p < 0.05$), and lignin concentration ($p < 0.01$). The narrow sense heritability estimates for biomass yield was very low (0.10), indicating a possible challenge to improve this trait. A genetic gain of 16.5% is predicted for biomass yield in each cycle of selection by recombining parental clones of 10% of superior progenies.

Keywords: biomass yield; genetic gain; genetic variation; half-sib; narrow sense heritability; switchgrass (*Panicum virgatum* L.)

1. Introduction

Switchgrass (*Panicum virgatum* L.) is a warm-season perennial grass native to the North American Prairie. Several uses of switchgrass have been reported which include forage for animals, soil stabilization for erosion control, and habitat for wildlife and migratory birds [1,2]. In the early 1990s, switchgrass was identified as a model herbaceous energy crop by the U.S. Department of Energy (USDOE) due to several desirable attributes including high biomass yield, relative ease to establish from seed, perennial growth habit, and adaptability to poor soil [1,3,4]. This has encouraged scientists to conduct extensive research to improve switchgrass as a bioenergy crop. The Renewable Fuel Standard (RFS-2) has mandated the annual production of 21 billion gallons of advanced biofuel by 2022 [5]. Cultivar breeding for improved biomass yield and lignocellulose components of a dedicated bioenergy crop such as switchgrass would contribute to achieving this target.

The natural populations of switchgrass are highly heterogeneous and heterozygous because of its outcrossing nature of reproduction mainly due to the S–Z system of gametophytic

self-incompatibility [6]. Switchgrass has evolved into two distinct ecotypes—lowland and upland [7,8]. Lowland ecotypes are spread across the southern region and adapted to a warmer and wetter climate, while upland ecotypes prevail in the northern region where the climate is relatively cold and dry [4,9]. A distinct morphological and cytological difference exists between these two ecotypes [10–12]. Lowland ecotypes are characterized by having thicker stems, being taller, and having high biomass yield compared to upland ecotypes. Based on chloroplast DNA polymorphism, switchgrass is also differentiated into either U or L cytotype [13]. The L cytotypes are tetraploids and associated with lowland ecotype; whereas, the U cytotypes are either tetraploids or octaploids and are associated with the upland ecotype [8]. A few intermediate types with an inconsistent chloroplast DNA polymorphism were also reported, which possibly resulted from natural hybridization and gene flow between upland and lowland ecotypes [8,14]. A cytological study revealed various ploidy levels among and within ecotypes that range from diploid (2n = 2x = 18) to dodecaploid (2n = 12x = 108) [10,11,15,16]. However, most of the switchgrass cultivars are either tetraploid (2n = 4x = 36) or octaploid (2n = 8x = 72). Lowland ecotypes are tetraploid, while upland ecotypes are predominantly octaploid, but a few tetraploids are also reported within upland ecotypes such as "Summer" [8].

The success of switchgrass as feedstock for biofuel production greatly depends on the improvement of biomass yield and its lignocelluloses composition such as cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are the positive contributors of ethanol recovery by the biorefinery process, whereas lignin is an inhibitory substance that prevents the access of enzymes during the conversion process. However, lignin is an energy-rich compound, with the energy content of approximately 25 MJ kg^{-1}, and could be a useful source of energy required for ethanol distillation [17]. The advancement in biotechnology provides a promising route to genetically modify and expedite switchgrass cultivar development for high biomass yield and ethanol recovery; however, the regulatory requirements limit its scope at least for several years in the near future. Conventional breeding has historically played a significant role in improving the genetic architecture of biomass yield and other desirable traits in important crops. Commonly practiced conventional breeding methods used in biomass yield improvement in outcrossing species such as switchgrass are: recurrent restricted phenotypic selection, half-sib progeny tests, among and within family selection, and recurrent multi-step family selection [18]. These breeding methods utilize additive genetic variation. However, the importance of heterosis has also been reported in switchgrass [19–22].

Several studies conducted in the past to improve biomass yield and agronomic traits in switchgrass were focused on capturing additive genetic variation [23–26]. Half-sib family selection is the most frequently used breeding method in switchgrass to capture additive genetic variation. A significant genetic variation has been reported for biomass yield and yield-related components in different half-sib populations [27–29]. These studies were performed in space planted nurseries with a plant to plant spacing ranging from 106 to 125 cm. It has been reported that studies conducted under a sparsely planted nursery have low prediction power for biomass yield compared to the densely planted nursery [30]. A recent study conducted in moderately high density planted nursery (plant to plant spacing: 30 cm and row to row spacing: 90 cm) validated the existence of a significant genetic variation among and within Alamo half-sib families [31]. Predicted gain per cycle of selection for a trait depended upon the amount of genetic variation in the population, heritability, intensity of selection, and the efficiency of mating system [18]. Previous studies suggest that biomass yield has been successfully increased by 20 to 30% to date at the rate of 1–2% gain per year using recurrent selection methods [25]. The narrow sense heritability of biomass yield has been reported to be low [28,29,31,32]. Low heritability of biomass yield suggests a potential challenge to improve this trait. The narrow sense heritability estimates of other agronomic traits such as plant height, stem thickness, tillering ability, and spring regrowth are reported to be moderately high to high [29,32]. On the other hand, the heritability of the major components of lignocellulose such as cellulose, hemicellulose, and lignin has not been extensively studied in switchgrass. Previous studies have reported the heritability of feedstock quality traits to be moderately high based on a family mean basis [33–35]; however, the estimates based on

individual plants are not available. An understanding of heritability of biomass yield and components of lignocellulose would be very helpful for breeders to predict the effectiveness of selection decisions.

A significant genotype × environment interaction has been well documented for biomass yield and other traits in switchgrass [36–39]. Lowland switchgrass produces high biomass yield; however, their survival becomes challenging in the northern region. It is reported that growing lowland switchgrass one hardiness zone north of their origin would cause a 9 to 17% reduction in biomass yield and survival [39]. Improving cold hardiness in lowland switchgrass along with yield to adapt in the northern United States is an important objective of switchgrass breeding [40]. "Kanlow", a high yielding lowland cultivar which was released in 1963 jointly by Kansas Agriculture Experiment Station (KAES) and the Agriculture Research Service (ARS) [41], has good adaptability from southern latitude to 40° N in North America, and it can tolerate some extent of the cold environment as compared to high yielding lowland cultivar Alamo. Therefore, Kanlow has great potential to be used in breeding for higher cold tolerance. In the past, most studies focused on studying genetic variation in lowland switchgrass have used Alamo or Alamo derived populations [23,27–29,31]. In contrast, not many studies were conducted on Kanlow or Kanlow derived populations, except a few studies which utilized Kanlow genetic background to generate hybrids with upland cultivar "Summer" [35]. This provides a good motivation to understand the extent of genetic variation in Kanlow and estimate genetic parameters such as heritability of important traits. The study reported here evaluated 54 half-sib families derived from the Kanlow population, and the evaluation was carried out under a moderately high plant-density (Plant spacing: 0.27 m^2 in current study vs. 1–2.25 m^2 in previous studies [27–30]). The objectives of this study were (i) to assess genetic variation for biomass yield and the components of lignocelluloses, (ii) to estimate the heritability of biomass yield, and (iii) to predict genetic gain per cycle of selection for biomass yield and the components of lignocelluloses in the Kanlow population.

2. Materials and Methods

2.1. Generation of Half-Sib Families

Kanlow half-sib (KHS) families of lowland switchgrass were generated in fall 2011. To generate KHS families over 200 genotypes were selected from a four-year-old sward of Kanlow switchgrass which was established in Fall 2007 at Holston Unit Farm of the University of Tennessee, East Tennessee Research and Education Center (ETREC) (35°58′32″ N; 83°51′25″ W). Individual plant selection was based on visual vigor at maturity. Open pollinated seeds were harvested separately from each of the selected plants. Half-sib families generated from phenotypically selected plants would help to check if phenotypic selection offered any genetic gain. Collected seeds were threshed, cleaned, and stored separately in envelopes. The KHS families which had enough seeds (approximately 200 seeds) were retained and advanced to germination. To break seed dormancy, seeds were treated with 100% household bleach (5.25% Sodium Hypochlorite) for 15 min, rinsed twice with tap water, and wet-chilled at 4 °C for one week [29]. Treated seeds were placed for germination on wet filter paper in Petri-dishes and incubated at room temperature (24 °C). Germinated seedlings of KHS families were transplanted in 72-well flats filled with greenhouse soil (Metromix 300, Griffin Greenhouse, Knoxville, TN, USA), and seedlings were raised in the greenhouse (25/15 °C day/night; 16 h light) for approximately 12 weeks. Finally, 54 KHS families which satisfied the number of seedlings required for replicated field testing were used in the experiment.

2.2. Field Evaluation

The KHS families were evaluated in the field at two locations in Tennessee, Holston Unit Farm of ETREC, Knoxville (35°58′42″ N; 83°51′28″ W), and Plateau Research and Education Center (PREC), Crossville (36°00′56.3″ N; 85°07′56.0″ W). Soil type in the Knoxville field nursery was Shady-Whitwell complex (fine-loamy, mixed, sub-active, thermic Typic Hapludults; fine-loamy, siliceous, semiactive, thermic Aquic Hapludults), and it was Lonewood loam (fine-loamy, siliceous, semiactive, mesic Typic

Hapludults) in Crossville. The experiment was conducted in a randomized complete block design with three replications at both locations. Seedlings of 54 KHS and Kanlow original population as check were transplanted in the field nursery at ETREC and PREC on 31 May and 7 June 2012, respectively. Each family in each replicate was planted in a 9-plant single-row plot, with the plant to plant spacing 0.3 m, and row to row spacing 0.9 m. Fertilizer was not applied during the establishment years. The plot was amended with 60 kg ha^{-1} N each spring during the post-establishment year. To keep weed pressure minimum, pre-emergence herbicide Dual II Magnum (Metolachlor; Syngenta Crop Protection, Inc., Greensboro, NC, USA) at the rate of 2.84 L ha^{-1} and Prowl H_2O (Perdamethalin; BASF Corporation, Research Triangle Park, NC, USA) at the rate of 3.31 L ha^{-1}, and post-emergence application of 2,4-D at the rate of 2.37 L ha^{-1} with surfactant at the rate of 1.18 L ha^{-1} were applied after a week of transplanting and during early spring in each post-establishment year. Holston Unit Farm, ETREC, Knoxville plots had been severely infested with Nutsedge (*Cyperus* spp.) during early growth stage; therefore, a post-emergence herbicide Accent (DuPont) at the rate of 18.9 g ha^{-1} mixed with crop oil (1% *v/v) was applied approximately 60 days after transplanting*.

2.3. Data Collection

No data were recorded during the establishment year, i.e., in 2012 (Year 1). The plots at both locations were mowed at the end of the growing season. Biomass yield data for 2013 growth (Year 2) were recorded on 14 Nov, and 19 at Crossville and Knoxville, respectively; while for 2014 (Year 3), biomass was harvested on Dec 14 at Crossville and on 22 Jan 2015, at Knoxville. Individual plots comprised of nine plants each were harvested as bulk. In 2013, five tillers were randomly sampled from five plants in each plot to estimate within family variation. The tiller samples of individual genotypes were dried and weighed separately. Other phenotypic data such as plant height at maturity were recorded in centimeters; only five plants per plot from two replications were measured. Stem thickness (1 = the smallest to 5 = the largest stem thickness) and tillering ability (1 = less than 10 tillers to 9 = more than 80 tillers) were also recorded at the same time from the same plants at both locations.

2.4. Feedstock Composition Analysis

Above ground plant tillers were collected just before biomass harvesting. Five tillers were randomly collected from each of five plants of a plot in two replications. Sampling was done individually in 2013. However, 2014 composition analysis samples were collected in bulk at the time of harvesting. Samples were oven dried and ground in a two-step process, coarse ground using Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA) and finely ground using a Cyclone grinder (UDY Corp., Fort Collins, CO, USA) to pass through 1 mm mesh. Ground samples were scanned using SpectraStar^{TM} Unity Scientific near-infrared spectroscopy (NIRS) platform to estimate the components of lignocelluloses—cellulose, hemicellulose, and lignin concentration. Cellulose concentration was calculated by subtracting lignin from ADF (Acid Detergent Fiber); whereas, hemicellulose concentration was computed by subtracting ADF from NDF (Neutral detergent fiber) [36,40].

2.5. Data Analysis

2.5.1. Variance Components

Data were analyzed using the MIXED model analysis (PROC MIXED) in SAS 9.4 (SAS Institute, Cary, NC, USA). Variance components for each trait were estimated using the restricted maximum likelihood (REML) method. In the data analysis model, location and year were considered as fixed effects, whereas replication and family were considered as random effects. Plot means (mean of 9 plants) were calculated for each year (2013 and 2014) and location (Knoxville and Crossville) to analyze genetic variation among KHS families. Within the KHS family, variation of biomass yield was calculated for the year 2013 using individual plant biomass data. Biomass weight of individual plants within a family plot was calculated by multiplying average tiller weight with total tiller count.

2.5.2. Comparison of Mean Performance of Traits

Biomass yield and other trait data were analyzed using the MIXED model analysis in JMP Pro 14 (SAS Institute, Cary, NC, USA). In the mixed model analysis, KHS, location, and year were considered fixed effects factors; and replication was considered a random effect factor. Least square means were obtained for each KHS family and Kanlow check. Any significant difference between the least square means was detected using Fisher's protected LSD ($p < 0.05$). The efficiency of phenotypic selection was tested by comparing group means of KHS and Kanlow check using an orthogonal contrast. Box plots to visualize biomass yield of KHS families were generated using SAS Enterprise Guide 7.1 (SAS Institute, Cary, NC, USA).

2.5.3. Narrow Sense Heritability

Narrow sense heritability of biomass yield, the components of lignocellulose, and other morphological traits were estimated on an individual plant basis by using variance components that were obtained from the analysis of variance. The equation used to compute the narrow sense heritability was based on equation described by Eberhart and Newell [42]:

$$h^2 = \frac{\sigma^2_A}{\sigma^2_P} = \frac{4 \times \sigma^2_{hs}}{\sigma^2_{hs} + \sigma^2_{hs \times Y} + \sigma^2_{hs \times L} + \sigma^2_{hs \times Y \times L} + \sigma^2_w}$$

where,

σ^2_A = additive genetic variance
σ^2_P = phenotypic variance
$\sigma^2_{hs} = \frac{1}{4} \times \sigma^2_A$ = variance among half − sib families
$\sigma^2_{hs \times Y}$ = vaiance due to family × year interaction
$\sigma^2_{hs \times L}$ = vaiance due to family × location interaction
$\sigma^2_{hs \times Y \times L}$ = vaiance due to family × year × location interaction
σ^2_w = variance associated with the difference in plants within a half – sib family

2.5.4. Phenotypic and Genetic Correlation

The phenotypic and genetic (additive) correlation was computed in SAS by using Proc MIXED restricted maximum likelihood (REML) method as described by Holland [43]. The estimation of the phenotypic and genetic correlation was based on the equation described by Miller et al. [44].

$$\text{Genetic correlation } \left(r_g\right) = \frac{\sigma_{g(\text{trait 1, trait 2})}}{\sqrt{\sigma^2_{g(\text{trait 1})} \times \sigma^2_{g(\text{trait2})}}}$$

$$\text{Phenotypic correlation } \left(r_p\right) = \frac{\sigma_{p(\text{trait 1, trait 2})}}{\sqrt{\sigma^2_{p(\text{trait 1})} \times \sigma^2_{p(\text{trait2})}}}$$

where,

$\sigma_{g(\text{trait 1, trait 2})}$ = the genetic covariance between trait 1 and trait 2
$\sigma_{p(\text{trait 1, trait 2})}$ = the phenotypic covariance between trait 1 and trait 2
$\sigma^2_{g(\text{trait 1})}$ = the genetic variance of trait 1
$\sigma^2_{g(\text{trait2})}$ = the genetic variance of trait 2
$\sigma^2_{p(\text{trait 1})}$ = the phenotypic variance of trait 1
$\sigma^2_{p(\text{trait2})}$ = the phenotypic variance of trait 2

2.5.5. Predicted per Cycle Genetic Gain

Predicted per cycle genetic gain (ΔG) from selection was calculated using the equation described by Nguyen and Sleper [45].

$$\Delta G = k\, c\, h^2\, \sigma_p$$

where,

k = the standardized selection differential (for 10%, k = 1.76: for 15%, k = 1.55)
c = parental control factor (for remnant seed, c = 1; for parental clone, c = 2)
h^2 = narrow sense heritability on an individual plant basis
σ_p = phenotypic standard deviation

3. Results

3.1. Variance Components of Biomass Yield and Morphological and Quality Traits

Variance components of biomass yield are presented in Table 1. A significant genetic variation ($p < 0.05$) among KHS families was observed for biomass yield across locations (Knoxville and Crossville) and years (2013 and 2014). The additive genetic variance is four times the variance among half-sib families in the absence of dominance and epistasis. On this theoretical ground, at least 9.7% of phenotypic variation among KHS families was contributed by the additive genetic effects for the combined data. Genetic variation for biomass yield among KHS was found to be wider within each location when analyzed separately ($p < 0.01$, Table 1, Figure 1). The additive genetic variation for biomass yield accounted for 22% and 19% of the total phenotypic variation of KHS families in Knoxville and Crossville, respectively. Our result showed that a large amount of variation was shared by the plants within family variation.

Table 1. Variance components and tests of fixed effects due to location and year for biomass yield (t ha^{-1}) in 54 Kanlow half-sib (KHS) families across locations (Knoxville and Crossville) and years (2013 and 2014).

Sources	Df	Knoxville	Crossville	Combined
		——————Variance Component——————		
KHS	53	1.67 **	2.26 **	0.96 *
Rep †/Rep [Location]	2 (4) ‡	0.04	1.77	0.90
KHS × Location	53	-	-	0.99 *
KHS × Year	53	0.44	0.13	0.30
KHS × Rep/Rep [Location]	106 (212) ‡	1.43 **	2.85 **	2.14 ***
KHS × Year × Location	53	-	-	-
KHS × Year × Rep [Location]	108 (216) ‡	1.68 ***	5.02 ***	3.28 ***
Plant [KHS] §	df	28.44 ***	45.53 ***	37.24 ***
		——————Test of Fixed Effects (F-Values)——————		
Year	1	379.32 ***	357.64 ***	612.52 ***
Location	1	-	-	87.56 **
Location × Year	1	-	-	10.01 **

* Significant at $p < 0.05$, ** Significant at $p < 0.01$, *** Significant at $p < 0.001$; † Rep, Replication; ‡ The df in parentheses indicates the df for combined locations; df (Knoxville = 268, Crossville = 214, Combined = 552); § Estimated from 2013 individual plant yield data which was obtained from five plants per plot using five tiller weight; two replications from Knoxville and one from Crossville.

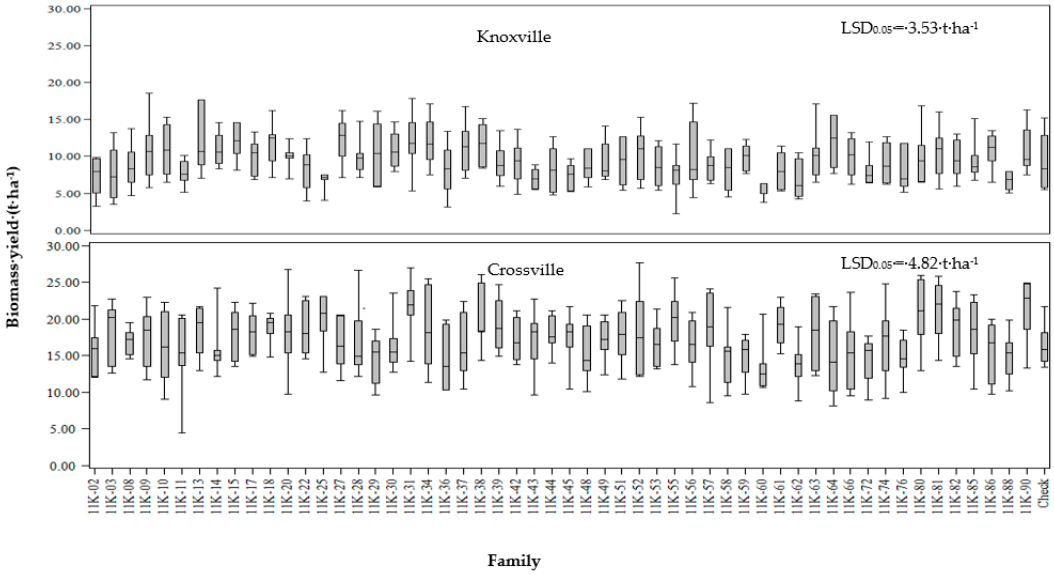

Figure 1. Variation in biomass yield of 54 Kanlow half-sib (KHS) families and Kanlow check at Knoxville and Crossville across 2013 and 2014.

Biomass yield was influenced by KHS × location interaction ($p < 0.05$) (Table 1, Figure 2). KHS interaction with year and location × year were not evident for biomass yield in this study. The fixed effect of locations ($p < 0.01$), year ($p < 0.01$), and location × year interactions ($p < 0.01$) were highly significant.

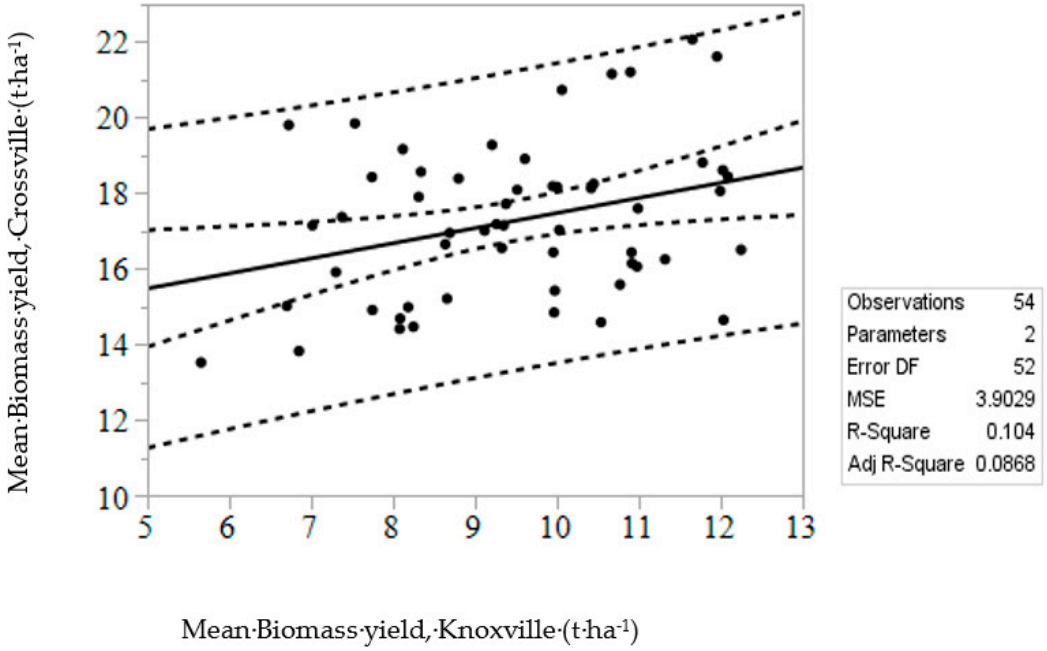

Figure 2. Mean biomass yield of 54 Kanlow half-sib (KHS) families demonstrating genotype × environment interaction across years (2013 and 2014). Deviation of dotted plots from the solid line in either direction showed the relative magnitude of genotype × environment interaction effect. The solid line showed the line of fit, dotted curved-line in either side of the line of fit showed the confidence curves for the fitted line, and dotted straight line in either side of the line of fit showed the confidence curves for an individual predicted value.

For morphological traits measured in this study, there was no significant variation observed for plant height, tillering ability, and stem thickness among KHS families. For feedstock quality traits,

a notable difference was observed in hemicellulose ($p < 0.05$) and lignin ($p < 0.01$) concentration, but there was no difference in cellulose concentration among KHS families (Table 2). Stem thickness showed a KHS × year interaction effect ($p < 0.01$). No other morphological and feedstock quality traits showed either a KHS × year or KHS × location interaction. For all measured traits, substantial variation was attributed to the plants within KHS ($p < 0.01$) (Table 2), which is also confounded with random error variation.

Table 2. Variance components and tests of fixed effects due to location and year for morphological and feedstock quality traits in 54 Kanlow half-sib (KHS) families across locations (Knoxville and Crossville) and years (2013 and 2014).

Sources	PH	TA	ST	CL	HC	LG
		Variance component				
KHS	5.39	0.04	-	0.01	0.14 *	0.06 **
Rep †/Rep [Location]	26.25	0.88	0.06	0.02	0.01	0.01
KHS × Location	-	0.01	0.01	-	-	-
KHS × Year	33.09	0.01	0.02	0.06	-	-
KHS × Rep/Rep [Location]	86.98 *	0.13 **	0.05 **	-	-	0.01
KHS × Year × Location	11.25	0.01	-	-	0.06	0.01
KHS × Year × Rep [Location]	192.08 ***	0.33 ***	0.11 ***	0.18 ***	0.61 ***	0.26 ***
Plant [KHS] §	485.28 ***	1.43 ***	0.48 ***	0.95 ***	1.56 ***	0.29 ***
		Test of fixed effects (F-values)				
Year	44.07 ***	0.08	139.08 ***	225.35 ***	88.62 ***	17.88 ***
Location	1.04	0.35	10.13	186.42 **	52.42 *	278.91 **
Location × Year	192.42 ***	0.30	215.93 ***	283.69 ***	68.50 ***	235.90 ***

* Significant at $p < 0.05$, ** Significant at $p < 0.01$, *** Significant at $p < 0.001$; † Rep, Replication; PH, Plant height in cm, from base of the plant to the tip of the longest tiller; TA, Tillering ability in the scaled score (<10 tillers = 1 to >80 tillers = 9); ST, Stem thickness in the scaled score (1 = the thinnest to 5 = the thickest); CL, Cellulose in the percentage on dry matter basis; HC, Hemicellulose in the percentage on dry matter basis; LG, Lignin in the percentage on dry matter basis; § Estimated from 2013 individual plant data which was obtained from five plants per plot using five tiller samples; two replications from Knoxville and one from Crossville.

The fixed year effects were highly significant ($p < 0.01$) for all measured traits except tillering ability; whereas, the location effect was only evident for feedstock quality traits cellulose ($p < 0.01$), hemicellulose ($p < 0.05$), and lignin ($p < 0.01$). All measured traits, except tillering ability, were also influenced by the location × year interaction effects.

3.2. Summary Statistics of Biomass Yield and Morphological and Quality Traits

Summary statistics of biomass yield and other measured traits are presented in Table 3. Biomass yield of KHS family at Knoxville ranged from 2.2 t to 18.5 t ha^{-1} with a mean value of 9.5 t ha^{-1}. At the Crossville location, biomass yield ranged from 4.4 to 30.4 t ha^{-1} with a mean value of 17.3 t ha^{-1}. Mean biomass yield of KHS family across locations (Knoxville and Crossville) and years (2013 and 2014) ranged from 9.6 to 16.9 t ha^{-1} with a mean value of 13.4 t ha^{-1}. Among 54 KHS families evaluated, nine produced higher biomass as compared to Kanlow check ($p < 0.05$). Biomass yield of 42 KHS families did not show any statistical difference than Kanlow check, whereas 3 KHS families produced significantly lower biomass yield.

Table 3. Summary statistics for measured traits of Kanlow half-sib (KHS) families and Kanlow check across four environments (Knoxville 2013–2014, and Crossville 2013–2014).

Trait †	BMY	PH	ST	TA	CL	HC	LG
				Knoxville			
KHS Mean	9.5	240.2	3.1	3.4	45.5	32.9	6.9
KHS Minimum	2.2	175.8	1.8	1.6	42.3	29.1	5.6
KHS Maximum	18.5	305.0	4.8	5.7	49.2	41.9	8.9
Kanlow check	9.3	249.7	2.8	3.3	45.4	32.5	6.9
$LSD_{0.05}$	3.5	48.5	0.6	1.0	2.5	1.7	1.2
CV%	34.5	11.9	17.5	22.2	3.6	4.1	11.9
				Crossville			
KHS Mean	17.3	245.6	3.8	4.2	43.5	34.6	5.4
KHS Minimum	4.4	209.2	2.4	2.0	41.6	31.6	4.1
KHS Maximum	30.4	293.6	5.0	6.9	45.9	37.2	6.7
Kanlow check	16.5	237.9	3.5	4.5	42.9	35.6	5.1
$LSD_{0.05}$	4.8	22.1	1.1	2.0	1.3	2.4	1.1
CV%	25.5	5.6	18.1	26.9	1.9	3.9	12.1
				Combined			
KHS Mean	13.4	242.8	3.4	3.8	44.6	33.7	6.3
KHS Minimum	9.6	226.0	3.0	2.9	43.9	32.6	5.6
KHS Maximum	16.9	262.0	4.2	4.5	45.4	35.1	6.9
Kanlow check	12.9	243.8	3.2	3.8	44.3	33.9	6.1
$LSD_{0.05}$	4.4	25.6	0.7	1.1	1.8	1.6	1.2
CV%	41.1	9.3	21.2	27.4	3.7	4.6	16.9

† BMY, Biomass yield (t ha^{-1}); PH, Plant height (cm); TA, Tillering ability (1–9 scale, 1 = the least, 9 = the most tillering plant); ST, Stem thickness (1–5 scale, 1 = the thinnest, 5 = the thickest); CL, Cellulose (% dry matter); HC, Hemicellulose (% dry matter); LG, Lignin (% dry matter).

Above ground height measured for KHS families ranged from 226 to 262 cm (Table 3). The plant height of KHS families was neither taller nor shorter than Kanlow check. The stem thickness score of KHS families ranged between 3 and 4.2; and only nine families had thicker stems than the Kanlow check, but none had a thinner stem. Mean tillering ability score of the KHS families ranged from 2.9 to 4.5, but none of the families had a significantly different score than Kanlow check.

Mean value of feedstock quality traits, cellulose, hemicellulose, and lignin were 44.6, 33.7, and 6.3, respectively (Table 3). Cellulose percentage ranged from 43.9 to 45.4, of which only two families had superior, but none had an inferior composition of cellulose compared to Kanlow check. Hemicellulose percentage ranged between 32.6 and 35.1. One KHS family had superior hemicellulose percentage, two families had inferior, and all other families were not statistically different. Lignin percentage ranged from 5.6 to 6.9. Six families contained higher lignin percentage, but the rest of the families had a statistically similar composition to the Kanlow check.

3.3. Efficiency of Phenotypic Selection

In this study, we used open-pollinated seeds that were harvested from the most vigorous plants in a four-year-sward of Kanlow switchgrass. The plants were identified based on visual vigor and overall phenotypic appearance at maturity. The performance of selected progenies was compared with the check to see if phenotypic selection offered any gain. For phenotypic selection to be effective, we expected that KHS would produce higher biomass yield as compared to the Kanlow check. Our result showed that KHS did not produce a statistically higher biomass yield (13.4 t ha^{-1}) compared to the Kanlow check (12.9 t ha^{-1}) (Table 3). The result suggested that phenotypic selection was not efficient to change the population mean in Kanlow from a single cycle of selection. Our result is consistent with previous studies conducted in an Alamo-derived population [31,46].

3.4. Heritability

Estimates of narrow sense heritability provide a foundation to predict genetic progress that could be achieved through cycles of selection. Out of seven traits considered in this study, only biomass yield, hemicellulose, and lignin showed the presence of genetic variation to make it possible to estimate heritability. The estimates of narrow sense heritability based on variance components are presented in Table 4. The estimates of narrow sense heritability for biomass yield, hemicellulose, and lignin were 0.10, 0.32, and 0.66, respectively.

Table 4. Estimates of narrow sense heritability of biomass yield and feedstock quality traits in Kanlow half-sib (KHS) families.

Parameter	Biomass Yield	Hemi-Cellulose	Lignin
σ^2_A	3.84	0.56	0.24
σ^2_P	39.49	1.76	0.36
h^2	0.10	0.32	0.66

σ^2_A = additive genetic variance, σ^2_P = phenotypic variance, h^2 = narrow sense heritability estimated on individual plant basis.

3.5. Genetic and Phenotypic Correlation

The genetic and phenotypic correlations of biomass yield with plant height, stem thickness, tillering ability, cellulose, hemicellulose, and lignin are presented in Table 5. Results showed biomass yield had positive phenotypic correlation with plant height (0.27 ± 0.06), and stem thickness (0.31 ± 0.06); low but positive correlation with tillering ability (0.19 ± 0.05), cellulose (0.16 ± 0.05), and lignin (0.15 ± 0.06); and very low negative correlation with hemicellulose (−0.08 ± 0.05). Further, our result showed that genetic correlation of biomass yield with plant height (0.54 ± 0.26) and stem thickness (0.75 ± 0.17) was moderate to moderately strong. Among quality traits, we observed a positive genetic correlation of biomass yield with cellulose concentration (0.39 ± 0.28) and lignin concentration (0.44 ± 0.20). The most desirable feedstock quality trait (cellulose) had a very strong positive genetic correlation with plant height (0.90 ± 0.42) and stem thickness (0.98 ± 0.33). Plant height and stem thickness were also positively correlated with biomass yield. Among traits considered in this study, stem thickness showed the strongest genetic correlation with both biomass yield (0.75 ± 0.17) and cellulose concentration (0.98 ± 0.33).

Table 5. Genetic (r_g) and phenotypic (r_p) correlation and their standard errors among biomass yield, morphological, and feedstock quality traits.

Trait	Biomass Yield	Plant Height	Stem Thickness	Tillering Ability	Cellulose	Hemicellulose
Plant height (r_g)	0.54 *** (±0.26)					
(r_p)	0.27 *** (±0.06)					
Stem thickness (r_g)	0.75 *** (±0.17)	0.67 *** (±0.27)				
(r_p)	0.31 *** (±0.06)	0.29 *** (±0.05)				
Tillering ability (r_g)	0.07 (±0.26)	−0.77 *** (±0.33)	−0.56 *** (±0.27)			
(r_p)	0.19 ** (±0.05)	−0.04 (±0.06)	−0.01 (±0.06)			
Cellulose (r_g)	0.39 *** (±0.28)	0.90 *** (±0.42)	0.98 *** (±0.33)	−0.19 ** (±0.37)		
(r_p)	0.16 ** (±0.05)	0.14 * (±0.05)	0.11 (±0.06)	0.07 (±0.05)		
Hemicellulose(r_g)	−0.17 ** (±0.29)	−0.25 *** (±0.38)	−0.39 *** (±0.31)	−0.24 *** (±0.34)	−0.54 *** (±0.30)	
(r_p)	−0.08 (±0.05)	−0.17 ** (±0.06)	−0.09 (±0.06)	−0.02 (±0.05)	−0.45 *** (±0.05)	
Lignin (r_g)	0.44 *** (±0.20)	0.78 *** (±0.27)	0.66 *** (±0.20)	0.01 (±0.26)	0.66 *** (±0.20)	−0.88 *** (±0.17)
(r_p)	0.15 * (±0.06)	0.17 ** (±0.06)	0.13 * (±0.06)	0.16 ** (±0.06)	0.60 *** (±0.04)	−0.51 *** (±0.04)

* Significant at $p < 0.05$, ** Significant at $p < 0.01$, *** Significant at $p < 0.001$; Biomass yield (t ha^{-1}); Plant height (cm); Tillering ability (1–9 scale, 1 = the least, 9 = the most tillering plant); Stem thickness (1–5 scale, 1 = the thinnest, 5 = the thickest); Cellulose (% dry matter); Hemicellulose (% dry matter); Lignin (% dry matter).

3.6. Predicted per Cycle Genetic Gain

Using the heritability computed in this study, we predicted selection gain for biomass yield (Table 6). With selection intensity of 15% and using remnant seeds of selected half-sib families (PC = 1), a gain of biomass yield is predicted to be 0.97 t ha^{-1} (7.2%). Using remnant seed and applying stringent selection pressure, i.e., 10%, a gain of biomass yield is predicted to be 1.10 t ha^{-1} (8.2%). With the selection intensity of 15% but using parental clones of selected half-sib families as a recombination unit, biomass yield can be doubled (a gain of 1.95 t ha^{-1} or 14.6%). The gain could be further enhanced when recombining only 10% of superior parental clones (a gain of 2.21 t ha^{-1} or 16.5%).

Table 6. Predicted per-cycle genetic gain (ΔG) for biomass yield, hemicellulose, and lignin concentration.

Selection Intensity	PC §	ΔG		
		Biomass Yield (t ha^{-1})	Hemicellulose (% Dry Matter)	Lignin (% Dry Matter)
10%	1	1.10 (8.2)	0.74 (2.2)	0.94 (15.5)
	2	2.21 (16.5)	1.49 (4.4)	1.89 (30.9)
15%	1	0.97 (7.3)	0.65 (1.9)	0.83 (13.6)
	2	1.95 (14.6)	1.31 (3.9)	1.66 (27.2)

§ PC, parental control (1 for the remnant seed of selected half-sibs, 2 for the parental clones of selected half-sib); $\Delta G = k\, c\, h^2\, \sigma_p$, where, k = the standardized selection differential (for 10%, k = 1.76: for 15%, k = 1.55), c = parental control factor, h^2 = narrow sense heritability on an individual plant basis, σ_p = phenotypic standard deviation; parentheses value indicates the gain in percentage.

Similarly, with a selection intensity of 15%, hemicellulose concentration could be improved by 2% using remnant seeds or 4% by using parental clones of selected half-sib families. With selection intensity of 10%, hemicellulose concentration could be enhanced by 2.2% using remnant seeds or 4.4% by using parental clones of selected half-sib families.

4. Discussion

Our study demonstrated a significant genetic variation among half-sib families and the plants within the family for biomass yield. Theoretically, a half-sib family accounts for one fourth of the total additive genetic variance, whereas three fourths of additive plus dominant variance are expected to be present within half-sib families [47]. On the basis this theoretical background, our results for among and within KHS family variation indicate that additive genes played an important role in biomass yield. The fact that a large amount of variation was shared by the plants within-family variation, non-additive genes might be abundant. However, we could not separate the total dominance variance present within half-sib families because estimation of error variance was not possible. Dominance variance could have been estimated if we had clonal replicates or if a different mating design was used, like the nested mating design used by Bhandari et al. [48]. Among feedstock quality traits assessed in this study, our results suggest that improvement of cellulose and hemicellulose is possible through among or within family selection. Depending upon the use of feedstock, lignin concentration could also be altered in a Kanlow population via classical breeding.

For all measured traits, substantial variation was attributed to the plants within KHS. Plants within a family were not a replication of a genotype in our experiment, and this variation among plants within a family which was confounded with random error variance could potentially mislead the true genotypic variation and selection decision. One way to improve the precision of estimating error variance would be by utilizing clonal copies of genotypes in the experiment. Casler and Brummer [49] specified clonal replication is not widely adopted by breeding programs because of time constraints and other resource burdens involved with it. Nevertheless, a breeder could invest resources in propagating clonal copies of genotypes, by tissue culture or other feasible methods, that would make it possible to estimate error variance. Estimation of error variance would lead to understanding the genetic effects

more precisely. It is advised to use at least a few clonal copies that would represent true replication of the genotype in the experiment.

Effects of environments and genotype × environment interaction has been well documented for biomass yield and other traits in switchgrass [36–39]. We observed a significant KHS × location interaction for biomass yield in our study which was possibly due to the varying response of the KHS family to the differences in temperature and precipitation. The two experiment sites are located within a similar latitude but at different elevations which likely contributed to the temperature difference. Crossville is located at an elevation of 580 m, as compared with 270 m for Knoxville. On average, the temperature in Crossville tends to be cooler than Knoxville. During the experiment period, the average annual temperature in Crossville was recorded to be 2.9 °C lower than Knoxville (based on the climate data obtained from the National Centers for Environmental Information) [50] (Table S1). Casler et al. [39] reported that temperature and photoperiod are important factors in determining switchgrass adaptation. This is likely due to the fact that the enzymatic activity linked to the metabolic pathways such as photosynthesis and respiration are affected by temperature fluctuation, which ultimately influences normal growth and development of switchgrass plants. A good example of temperature effect on switchgrass is that onset of winter (killing frost) arrests growth and imposes dormancy in the rhizomes [51]. Subsequently, the rise of temperature in the following spring helps to break dormancy and drives above-ground growth. However, if the genotype is sensitive to low temperature, spring frost could push back the regrowth in early breaking genotypes. Genotypes with some extent of cold tolerance would continue to grow as enzymatic activity would not be affected by reduced temperature during spring. Our results revealed that some families are less affected by the differences in temperature and produce consistently higher biomass across locations (Figure 1). Additionally, Crossville received higher precipitation (4633 mm) than Knoxville (4185 mm) during the experiment period from 2012 to 2014. The trends in precipitation were even more dissimilar between Knoxville and Crossville during the active growing season (April to September) of switchgrass. During the establishment year (2012), Crossville received 152 mm less precipitation than Knoxville. Conversely, in 2013 and 2014, Crosville received 95.5 mm and 285.1 mm more precipitation than Knoxville. Hui et al. [52] reported that increased precipitation significantly enhances biomass of switchgrass by stimulating leaf photosynthesis. We observed that a yield gap of above ground plant biomass of KHS family between Knoxville and Crossville increased to 8.3 t ha^{-1} in 2014 rather than 5.3 t ha^{-1} in 2013 (data not presented). We speculate that variation in precipitation is one of the important factors that could have affected biomass accumulation in some switchgrass families by interfering with photosynthetic activity. Genotype × location interaction was such a high component in the current study that the biomass yield at Knoxville explained only 10% of the variation in biomass yield at Crossville (Figure 2). Our result is in agreement with previous studies for a genotype × location interaction [29,38], validating the necessity of multi-location trials for making selection decisions to improve biomass yield in switchgrass. Furthermore, the existence of genotype × location interactions emphasizes the development of regionally adapted cultivars to maximize genetic gain in the target region [25,53].

Our result showed that the fixed effect of locations, year, and location × year interactions were highly significant. Among all, the magnitude of the year effect was the largest ($p < 0.01$) on biomass yield variation, which is not surprising for perennial grasses such as switchgrass. It is well documented that switchgrass reaches full yield potential only by the third year of growth because maximum energy is diverted for root development during the establishment years [4].

Phenotypic selection was not effective in our study, suggesting that selection solely based on individual plant vigor would not be efficient for complex traits like biomass yield. In our selection, the individual genotype variation in sward could have been associated with environmental variation such as topography, soil texture, soil nutritional status, and other microenvironments within sward. Additionally, Brown et al. [54] suggests that phenotypic selection would not be efficient for the traits having low heritability. In this study, genetic variation for biomass yield was observed; but within-family

variation was very high, leading to low heritability. Further, Falconer and Mackay [55] described that pollen from undesirable plants could dilute the desirable alleles in the selected plant. Planting of the selected plant into an isolated polycross block to generate plant materials could produce favorable recombinants. However, this process would significantly delay the selection cycle, thus reducing genetic gain per year. Casler [56] recapped that most field-based phenotypic selection in forage crops requires 2 years per cycle. If phenotypic selection is the breeder's interest, then it could be performed by applying some restriction as practiced by Burton [57] in Pensacola bahiagrass (*Paspalum notatum* var. *saure* "Parodi").

We computed narrow sense heritability for biomass yield and found it to be low. Low heritability for biomass yield on an individual plant basis was observed due to the fact that the quantitative traits measured on individual plants produced a large residual effect. Previous studies conducted using Alamo-derived populations reported a similar estimate of heritability for biomass yield [28,29,31,32]. Because narrow sense heritability is a function of additive variance, genotype × environment interaction, and random error, changes in the magnitude of any of these variances would alter heritability. Low heritability of biomass yield in this study could be associated with a very high genotype × environment interaction and random error. This is reflected in the higher heritability estimates within each location (Knoxville $h^2 = 0.22$ and Crossville, $h^2 = 0.19$). Low heritability estimates for biomass yield suggests possible challenges to improve this trait. For difficult traits like biomass yield, selection based on a secondary trait which is highly heritable and correlated with the trait of interest could be very effective to make positive progress.

Breeders often rely on indirect selection if primary traits of interest are very difficult or expensive to measure because of several factors such as polygenic nature, low heritability, and the presence of genotype × environment interactions [55]. Success of indirect selection relies on the high heritability of the secondary trait, ease of selection, and correlation with the primary trait. Therefore, knowledge of phenotypic and genetic correlation among different traits is important in plant breeding. A phenotypic correlation between two traits illustrates the correlation of both genetic and environmental effects. The genetic correlation, on the other hand, indicates that the genes that contribute to the traits are usually co-inherited, and is more important to the breeder for improving difficult traits through indirect selection. Bhandari et al. [29] and Sykes et al. [30] reported a stronger positive correlation of biomass yield with plant height and stem thickness—twice as much as the phenotypic correlation we found in this study. Furthermore, tillering ability was found to have a poor correlation with biomass yield in our study, contradicting the result published by Das et al. [27]. However, as mentioned earlier, it should be noted that their results were based on the study conducted in a space planted nursery, which has low prediction power as compared to ours which had higher plant density. Genetic correlation of biomass yield with plant height and stem thickness was moderate to moderately strong in the present study, suggesting that the combination of these two traits have potential to serve as good candidates for indirect selection to improve biomass yield. However, breeders need to be vigilant as we were not able to estimate heritability of neither plant height nor stem thickness in our study because of non-significant genetic variation among KHS family. We assume that non-significant genetic variation of these two traits could be associated with the small population size in this study, which was not enough to detect the low level of natural variation present in the Kanlow population which may not be the case if a large population had been used. For example, Jahufer and Casler [34] reported an increment in heritability estimate for biomass yield using a bigger population size. Past studies in Alamo derived populations presented significant genetic variation and moderate heritability for plant height and stem thickness [29,48].

We observed a positive genetic correlation of biomass yield with cellulose and lignin concentration. From our results, it is evident that improving biomass yield alone would lead to an increase in cellulose and lignin concentration in the Kanlow population. To make the feedstock industry more profitable, the ideal breeding procedure in switchgrass is to improve biomass yield in parallel with improving the quality traits. Positive genetic correlation between biomass yield and lignin in our study is in agreement

with Jahufer and Casler [34]. Conversely, Edmé et al. [35] reported a negative correlation between biomass yield and lignin in their studies (−0.33 ± 0.22). The negative correlation between biomass yield and lignin, as reported by Edmé et al. [35], may be a result of the upland switchgrass genetic background involved in their study. Depending upon the use of biomass feedstock, i.e., for ethanol recovery, pyrolysis, or combustion, breeders may choose to modify cellulose and lignin by improving biomass yield via. classical breeding. Improvement of biomass yield in conjunction with high cellulose or lignin concentration in Kanlow can be achieved by selecting taller and thicker stemmed plants; however, the breeder needs to look for additional traits (individual or combination of two or more traits) in future studies such as "leafy tillers", "plant posture", "thicker stem with high tillering ability", or "thicker stem with high leaf number" to find traits that have greater genetic correlation with biomass yield for use in indirect selection. Our result suggests that maximum genetic gain is possible for each biomass yield and hemicellulose in Kanlow switchgrass by recombining clonal parents of 10% superior families.

5. Conclusions

The results of the current study showed the existence of genetic variability for biomass yield both among and within the Kanlow population. Selection based on single plant performance may not be effective to improve biomass yield. Accumulation of favorable additive genes to improve biomass yield could be practiced by employing rigorous family performance-based selection or among and within family selection [49]. The results also signify that genes controlling biomass yield are highly influenced by genotype × location interaction, suggesting the necessity of a target region based multi-location experiment for making the selection decision. Indirect selection based on morphological traits such as stem thickness may improve both biomass yield and feedstock quality.

The magnitude of half-sib family variance is much smaller than within-family variance, and heritability is low for biomass yield which suggests that non-additive genes may be abundant. Currently, the genetic gain achieved by switchgrass breeders mostly relies on additive genes. Further study using clonal replicates in a half-sib mating system or by choosing a different mating design, such as a diallel or nested design (North Carolina Design I), would be helpful for partitioning of additive and dominance variance. Understanding of both additive and non-additive genes would be valuable to adopt an ideal breeding method for the improvement of Kanlow switchgrass. The development of high yielding Kanlow switchgrass cultivars along with better feedstock quality and a wide range of adaptability has great potential to sustain future bioenergy requirements.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/12/1845/s1, Table S1: Climate data of Knoxville and Crossville during 2012, 2013, and 2014.

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