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Genetic Analysis of Yield and Quality Traits in Switchgrass Based on Population Crosses

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Abstract: Obtaining greater genetic gains, particularly for biomass yield, requires a good understanding of the gene action governing the inheritance of traits with economic importance in switchgrass (*Panicum virgatum* L.). Individual genotypes from three different accessions were crossed in single-pair matings with reciprocals to assess the relative importance of additive to nonadditive genetic variation and the potential of using inter-ecotypic crosses to improve dry matter yield (DMY), in vitro dry matter digestibility (IVDMD), lignin content (ADL and KL), and ethanol yield (ETOH). Crosses and four reference populations were planted in a randomized complete block design with eight replications of single family-rows plots, with five-plants each and 1 m spacings. A linear mixed model was applied as per the restricted maximum likelihood method, integrated with a pedigree tracing back to the original founders of these parental populations, and augmented with the designation of four genetic groups. Variation due to SCA (specific combining ability) was predominant for all traits, contributing from 20% to 57% of the total phenotypic variation and with Baker's ratios (GCA/SCA) varying from 0.003 to 0.67. Heritability values calculated at the fullsib-family mean level were moderate to very high. Variation due to GCA (general combining ability) was detected with a lesser significance for DMY and ETOH. A reciprocal GCA effect was present in the form of maternal inheritance for DMY, suggesting the use of the highest biomass-yielding parent as female in inter-ecotypic breeding. Selecting and deploying fullsib families, deploying clonal hybrids, and adopting an introgression breeding approach are all possibilities available to switchgrass breeders to exploit the complementary genes from this germplasm and capitalize on the non-additive genetic variation present in these crosses.

Keywords: biomass; breeding values; ethanol; GCA; lignin; pedigree; reciprocal; SCA; switchgrass



Citation: Edmé, S.; Mitchell, R. Genetic Analysis of Yield and Quality Traits in Switchgrass Based on Population Crosses. *Agronomy* **2021**, *11*, 2220. <https://doi.org/10.3390/agronomy11112220>

Academic Editor: Federica Zanetti

Received: 28 September 2021

Accepted: 29 October 2021

Published: 2 November 2021

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1. Introduction

Switchgrass (*Panicum virgatum* L.) is an economically and ecologically important crop species in the USA, providing forage for livestock, for prairie restoration, for landscaping, and potentially for biofuel production [1]. The interest in breeding switchgrass for bioenergy increased markedly in the 1990s with the realization that (1) it is indigenous to the USA, (2) it has high yield potential, (3) it yields particularly better than field crops in marginal environments [2], (4) it has great potential for carbon sequestration due to its perennial nature and deep root systems [3], and (5) it has already existing and thriving breeding and research programs. The USDA-ARS breeding program in Lincoln NE released the first bioenergy cultivar as “Liberty” after one cycle of selection in an upland (“Summer”) × lowland (“Kanlow”) population [4].

Breeding switchgrass is ordinarily and primarily based on exploiting additive genetic variation in halfsib recurrent selection protocols within ecotypic populations adapted for a specific region of the country [5]. Practically, parents are collected from wild stands in the prairies, tested, and selected for crossing in wind-pollinated nurseries. Seed is collected from the female tassels and tested as halfsib (open-pollinated) families. Selection is first

practiced between and then within families in the progeny tests to obtain parents of the next generation. Recently, genetic analyses were introduced into the selection process to estimate breeding values of parents and progeny according to the restricted maximum likelihood method and the animal model approach [5,6]. Deployment of seed of the new cultivar is ultimately produced by wind/mass pollination after at least three cycles of multiplication without selection.

Additive genetic variation was reported to be present and ample to improve biomass and quality traits in switchgrass [5,7]. Other sources of variation (e.g., non-additive as dominance and/or epistasis) have not been fully examined for potential use as breeding and deployment strategies. Non-additive (dominance or epistatic) genetic variation, derived from allelic interactions within and between loci, can be exploited by way of biparental crosses using parents with the highest breeding values. The possibility exists also to select on the total genetic variation (additive + dominance + epistasis), pending the availability of a high-throughput protocol for obtaining clones from tissue culturing for vegetative propagation [8]. A rigorous assessment of the mode of inheritance of the objective traits (biomass, lignin content, ethanol yield, and disease resistance) selected for in switchgrass is greatly needed to understand how to maximize gains from breeding and selection.

Having multiple traits under selection in a breeding program complicates the breeder's strategies when some of the traits are under different genetic controls and adverse genetic correlations do not lean towards simultaneously maximizing gains in the traits. In this breeding program, biomass yield and other quality traits (digestibility, lignin content, ethanol yield, and disease resistance) are used as both selection and objective traits for forage or bioenergy (combustion and lignocellulosic) breeding goals. Biomass yield is largely emphasized in breeding switchgrass populations for bioenergy to increase its desirability to both growers and ethanol producers. Originally, switchgrass was bred for forage, and improving quality (high in vitro dry matter digestibility, lower lignin content) was pursued with higher intensity than biomass yield, probably due to having adverse genetic correlations. Biomass yield was also reported to have adverse genetic correlations with quality traits (low lignin content and ethanol production) when targeting the lignocellulosic industry and not so when the combustion platform is the goal [5]. To date, however, no specific study has addressed the relative abundance of additive and dominance (or non-additive = dominance + epistasis) genetic variation to the total genetic variation in switchgrass breeding populations, particularly when it comes to improving biomass yield.

Diallel analyses are design tools used by breeders to investigate and elucidate the inheritance mode of traits in plants [9]. Pair-plant crosses are made to study how the parents transmit genes to their progeny, either by positively combining their alleles irrespective of the other parents involved in the crosses (general combining ability), and/or by requiring specific crosses or parents to produce superior progeny (specific combining ability). The direction of the cross may be important depending on the trait when superior progeny are generated only if one of the two parents is used precisely as the female and not as the male. The animal model [10], an approach that integrates the pedigree-derived relationship matrix into the genetic analysis, is being increasingly adopted by plant breeders to improve accuracy and increase gains from a selection [11]. Accounting for the relationships among parents and progeny allows prediction of breeding values to be made and compared across generations, even for non-phenotyped base parents and individuals [12]. This approach was applied to this diallel dataset by additionally differentiating the genetic groups represented by the parents, which have different origins, have received different selection pressures, and have different allele frequencies and different levels (or generations) of genetic merits. To remove bias from pre-selection, considering the genetic grouping formed by parents, progeny, and ancestors of this population is expected to provide a reliable estimate of the additive genetic variance and consequently heritability to predict response to selection [13].

Appraising the value of inter-ecotypic crosses is a key component of the Nebraska-based USDA-ARS grass breeding program. Switchgrass breeding can benefit from intercrossing the genetic resources available, thus exploiting complementary gene action, heterosis for hybrid breeding and deployment, and introgression breeding. In that context, biparental crosses were made between plants derived from three indigenous tetraploid populations of switchgrass, with two classified as lowland and the other one as upland ecotypes. This study gave an opportunity to assess the relative importance of nonadditive to additive genetic variation and, consequently, explore if other breeding strategies (than for general combining ability) need to be considered for the traits targeted in switchgrass. The primary objectives of this study were: (1) to evaluate the relative importance of additive to non-additive genetic variance using a population diallel supported by a pedigree that integrates the three parental populations as genetic groups, and (2) to assess the value of reciprocal crosses in the breeding strategy involving biomass yield, lignin content, ethanol yield, and in vitro dry matter digestibility.

2. Materials and Methods

2.1. Genetic Materials and Experiment

Four tetraploid (4×) populations of switchgrass were used to make single pair-plant crosses and create fullsib families among accessions. The individual plants were selected from an upland cultivar ('Summer'), a lowland accession (King's County 'PI 19064231') from New York, and two advanced genetic lines derived from intra-population improvement programs within 'Kanlow' (a lowland cultivar) (Table 1). The Summer line (SL) was an advanced generation under selection for late maturity. The two Kanlow lines were from two separate breeding objectives, one being the target for early maturity and the other for high cellulose content and biomass yield. These two lines were kept separate in this analysis to properly identify the value of each in an outcrossing program as the allele/ gene frequencies might have changed with selection. All three lines were managed with a halfsib recurrent selection protocol.

Table 1. Description of the switchgrass parental and reference populations and number of crosses.

| Populations | Origin | Ecotype | Type | Selection for | Name | Coding |
|----------------|--------------|---------|---------------|----------------------|------------------|--------|
| PI 19064231 | New York | Lowland | Wild | None | Kings County | KC |
| Summer | Great Plains | Upland | Breeding line | Late maturity | Summer Late | SL |
| Kanlow | Great Plains | Lowland | Breeding line | Early maturity | Kanlow Early | KE |
| Kanlow | Great Plains | Lowland | Breeding line | Cellulose; bioenergy | Kanlow Bioenergy | KB |
| Crosses | | | | | | |
| KE × KC | | KC × KE | | | | |
| KE × SL | | SL × KE | | | | |
| KC × SL | | SL × KC | | | | |
| KB × KC | | KC × KB | | | | |
| KB × SL | | SL × KB | | | | |
| KB × KE | | KE × KB | | | | |

Fourteen to 20 plants per population were paired to make reciprocal crosses (i.e., inter-ecotypic) in the greenhouse at the USDA-ARS grass breeding program in Lincoln, NE. Self-incompatibility in switchgrass facilitates obtaining reciprocal crosses by separately collecting seed from each panicle of the paired male and female parents [14]. In total, 56 fullsib crosses were designed (28 paired-plants × 2) to produce seedlings in the spring of 2013. With only one clone available for each individual plant, a parent was involved only in a single-pair mating with reciprocal. The diallel was constructed on a population basis. Switchgrass being a polyploid, each fullsib seedling derived from a cross represents a different genotype, sharing several alleles in common with its sibs but inheriting a distinct allele mix from the two parents due to Mendelian sampling. Seedlings were germinated and cared for in the greenhouse from January to June of 2013 and subsequently transplanted

to the field according to a randomized complete block design with eight replications of the families. The seedlings were planted in single family-row plots at a rate of 5 plants per row, spaced 1.1 m apart within and across rows. Fertilization, herbicide spraying, and plot maintenance were in accordance with the protocol developed for perennial grasses in the breeding program [4]. Control plots of six reference populations were planted also as seedlings from seeds stored in a cold room: Kanlow, Summer, a Summer line selected for late maturity, a Kanlow line selected for winter survival, King's County, and a synthetic derivative of the first cycle of a Summer x Kanlow population. Four of these populations are the founders of the parent plants used to make the crosses.

2.2. Data Collection

Data were not collected the first year of field establishment as not enough biomass was available to be significant in analyses. Plant samples were collected after a killing frost in 2015 and 2017, transported to the lab for drying, weighing, grinding, and processed for lab analyses. Biomass of the field experiment was machine-harvested in 2015 on a plot basis and in 2017 three replications were harvested on a plot basis and one on an individual basis. A self-propelled flail harvester (Carter Manufacturing, Brookston, IN, USA) weighed the plots automatically and the weights were later converted to dry matter yield (DMY) per hectare using the dry weights of the samples collected prior to harvest. Lignin content (as an acid detergent or ADL and Klason or KL), in vitro dry matter digestibility (IVDMD), and ethanol yield (ETOH) were predicted from equations developed from near-infrared reflectance spectrometry (NIRS) using the ground samples [15]. In this breeding program, ADL is used in selection for a forage objective and KL for a bioenergy objective. Since the parents were selected from populations under improvement for forage and bioenergy, two NIRS predictions were made based on two equations: one for forage using DMY, ADL, and IVDMD and the other for bioenergy using DMY, KL, and ETOH.

2.3. Statistical Analyses

Univariate statistical analyses of the traits were carried out using the stand-alone software ASReml v4.2 [16]. The following linear mixed model was fitted to the data applying the restricted maximum likelihood method to extract the variance components:

$$Y_{ijklm} = \mu + L_i + B_j + G_k + G_l + S_{kl} + R_{lk} + LG_{ik} + LG_{il} + LS_{ikl} + LR_{ilk} + w_{ijklm} + \varepsilon_{ijkl} \quad (1)$$

where Y is the vector of observations on the traits of interest; μ is the overall mean; L is the fixed effect of years; B_j is the fixed effect of the j th replication within a year; G_k and G_l are the random additive genetic effects (i.e., general combining ability or GCA) of the k th female and l th male parents; S_{kl} is the random non-additive genetic effect of the cross (i.e., specific combining ability or SCA) involving the k th female and l th male parents; R_{lk} is the random reciprocal effect for the cross involving the l th female and k th male parents; LG_{ik} and LG_{il} are the random interaction effects between GCA of the female and male parents with years; LS_{ikl} is the random interaction effect between SCA of the cross with years; LR_{ilk} is the random interaction effect between the lk reciprocal cross and i year; w_{ijklm} is the random residual variation among individual plants within a plot, and ε_{ijkl} is the random residual variation among plot mean values.

The relationships existing within and among the parental populations were modeled in the analysis by integrating the pedigree-derived relationship matrix. Three genetic groups were defined as pertaining to the Kanlow, Summer, and King's County lineages and superimposed on the diallel pedigree to account for the differential origin and structure of the original populations and of their F1 progeny derivatives under selection. Lines were added in the pedigree to reflect the intra-population breeding history and to assign the parent plants in the diallel to their respective genetic groups, tracing back to the founder populations (as phantom parents). Accounting for the relationships among the parents of the diallel allows for the specific variance-covariance structure of the parents and, that way, assured a reliable estimation of the genetic effects [13]. As the parental populations were

included in the experiment as references, the analyses were performed on the complete phenotypic data collected on checks and fullsib progeny.

Estimates of narrow (h^2_N) and broad-sense (H^2_B) heritability were obtained for each trait at the fullsib family mean and within fullsib-family mean level to gauge the degree of inheritance and to examine potential breeding strategies. This breeding program usually follows a two-step strategy that first selects the best families/crosses and finally selects the best individuals within these families. To derive the (h^2_N) and (H^2_B) values, the following equations were used:

$$\sigma^2_P = 2 \sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_R + \sigma^2_{LG} + \sigma^2_{LS} + \sigma^2_{LR} + \sigma^2_w + \sigma^2_e \quad (2)$$

as the total phenotypic variance;

$$\sigma^2_{FS} = 2 \sigma^2_{GCA} + \sigma^2_{SCA} + \sigma^2_R + 2 \sigma^2_{LG}/\ell + \sigma^2_{LS}/\ell + \sigma^2_{LR}/\ell + \sigma^2_e/\ell r + \sigma^2_w/\ell r n \quad (3)$$

as the variance of fullsib family means;

$$\sigma^2_{FSw} = \sigma^2_P - \sigma^2_{FS} \quad (4)$$

as the variance of within fullsib-family means.

With ℓ being the number of years, r the number of replications, and n the number of plants/seedlings within a plot;

$$h^2_{FS} = 2 \sigma^2_{GCA} / \sigma^2_{FS} \quad (5)$$

for estimating h^2_N at the fullsib-family mean level;

$$H^2_{FS} = (2 \sigma^2_{GCA} + 3 \sigma^2_{SCA}) / \sigma^2_{FS} \quad (6)$$

for estimating H^2_B at the fullsib-family mean level;

$$h^2_{wfs} = 2 \sigma^2_{GCA} / \sigma^2_{FSw} \quad (7)$$

for estimating h^2_N at the within fullsib-family mean;

$$H^2_{wfs} = (2 \sigma^2_{GCA} + 3 \sigma^2_{SCA}) / \sigma^2_{FSw} \quad (8)$$

for estimating H^2_B at the within fullsib-family mean level.

The analyses started with implementing the full model with all parameters included. However, depending on the trait being analyzed, not all the components were estimable, at times equating 0 and creating convergence issues. These parameters were then removed from the finally accepted model which was checked for better fit before acceptance based on the change in the log-likelihood and the Akaike information criteria between the models. Standard errors of the genetic parameters, including heritability, were approximated using the Delta method [17]. The significance of the genetic parameters was determined based on the ratio of their variance estimates to the associated standard error.

The breeding values of parents (including founders) and progeny were predicted. The percentage contributions of GCA, SCA, and reciprocal components to the total phenotypic variation were also calculated. Of great interest is the relative importance of GCA (additive) to SCA (non-additive) genetic variation in guiding the breeding program towards integrating a fullsib recurrent selection strategy into its routine as compared to a pure halfsib selection method. The Baker's ratio was calculated as:

$$2\sigma^2_{GCA} / (2 \sigma^2_{GCA} + \sigma^2_{SCA}) \quad (9)$$

to gauge this relative importance.

3. Results

3.1. Genetic Parameters for Biomass (Dry Matter) Yield

Biomass yield is one of the traits in switchgrass that breeders struggle to improve over time, with the reason being a combination of its negative genetic correlation with quality traits (increased IVDMD and reduced lignin content) when breeding for forage and of more selection emphasis given to quality traits. For bioenergy breeding, increasing biomass yield has even greater importance to foster switchgrass competitiveness with other bioenergy crops, such as willow (*Salix spp.*) or sugarcane (*Saccharum spp.*). The inheritance of the trait was never questioned with ample additive genetic variation reported in genetic studies [5,6].

For biomass yield, most of the genetic variation was underpinned by the non-additive component and, to some extent, by the differences in the (reciprocal) GCA effects of a parent when used as a female or a male (Table 2). Non-additive genetic variation contributed 46% to the total phenotypic variation and reciprocal GCA contributed 7%. There were significant differences in the crosses between replications: with switchgrass being a polyploid, each of the individual seedlings or plants in the family plot represents a different genotype, even though they are fullsib progeny of two parents. Therefore, every family plot is a mix of different genotypes that potentially experienced dissimilar variation across the field. As a result, the crosses did not perform consistently from one replication to the next, and it is not possible to specify if that was due to micro-level variation across the field or due to the sampling of seedling genotypes. The additive genetic component was detected as a form of maternal effect, indicated in Table 2 as an interaction between GCA and reciprocal effects. There was no interaction between GCA and years of testing, neither between crosses and years.

Table 2. Heritability values, Baker's ratios, and significance of variance components obtained from parental crosses derived from three switchgrass ecotypic populations.

| Sources of Variation [†] | DMY [‡] | σ^2/SE [§] | IVDMD | σ^2/SE | ADL | σ^2/SE | KL | σ^2/SE | ETOH | σ^2/SE |
|-----------------------------------|------------------|----------------------------|--------|---------------|--------|---------------|---------|---------------|--------|---------------|
| GCA | 0.16 | 0 | | | 0.247 | 0.14 | 27.356 | 0.91 | 5.069 | 1.54 |
| Rep.GCA | - | - | 25.30 | 1.06 | 0.224 | 0.58 | 1.424 | 0.24 | - | - |
| Year.GCA | 0.00 | 0 | 22.81 | 1.53 | 2.628 | 1.38 | 18.304 | 1.03 | 0.249 | 0.37 |
| GCA.Recip | 0.85 | 2.26 | 0.00 | 0 | - | - | 1.286 | 0.27 | - | - |
| Year.GCA.Recip | - | - | - | - | - | - | 0.00 | 0.00 | - | - |
| Year.Rep | 0.07 | 0.53 | 49.24 | 1.63 | 0 | 0.00 | - | - | - | - |
| SCA | 3.01 | 2.78 | 43.61 | 1.43 | 6.575 | 2.19 | 53.570 | 1.13 | 5.011 | 1.41 |
| SCA.Reci | - | - | - | - | - | - | 48.652 | 1.02 | 1.855 | 0.68 |
| Rep.SCA | 1.59 | 2.56 | 10.23 | 0.17 | 1.809 | 1.24 | 13.665 | 0.55 | 3.498 | 1.85 |
| Year.SCA | 0.23 | 0.57 | 0.00 | 0 | 1.013 | 0.52 | 23.914 | 0.78 | 0.404 | 0.26 |
| Year.SCA.Recip | - | - | - | - | 0.630 | 0.39 | 30.044 | 0.72 | 5.340 | 1.83 |
| Year.Rep.SCA | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.726 | 0.31 |
| Residual | 10.10 | 13.3 | 703.18 | 13.86 | 25.208 | 13.26 | 467.532 | 13.2 | 22.476 | 10.9 |
| h^2_{FS} ^Φ | 0.06 | | 0.00 | | 0.06 | | 0.20 | | 0.38 | |
| H^2_{FS} | 0.59 | | 0.28 | | 0.59 | | 0.39 | | 0.57 | |
| h^2_{wfs} | 0.03 | | 0.00 | | 0.03 | | 0.12 | | 0.38 | |
| H^2_{wfs} | 0.88 | | 0.19 | | 0.88 | | 0.46 | | 0.93 | |
| Baker's Ratio | 0.10 | | 0.003 | | 0.10 | | 0.51 | | 0.67 | |

[†] GCA = general combining ability; SCA = specific combining ability; Recip = reciprocal; Rep = replication; [‡] DMY = dry matter yield; IVDMD = in vitro dry matter digestibility; ADL = acid detergent lignin; KL = Klason lignin; ETOH = ethanol yield; [§] Variance component (σ^2) divided by standard error (SE); ^Φ narrow-sense (h^2) and broad-sense (H^2) heritability calculated at the fullsib-family (FS) mean and within fullsib-family (wfs) mean levels, respectively.

Narrow-sense heritability, measured at the fullsib-family mean level, for biomass yield was low (0.06 ± 0.02) and broad-sense heritability was high (0.59 ± 0.23). At the within fullsib-family mean level, these two heritability values were $0.03 \pm$ and 0.88 ± 0.16 , respectively.

3.2. Genetic Parameters for Quality Traits

3.2.1. In Vitro Dry Matter Digestibility (IVDMD)

For IVDMD, non-additive genetic variation was more important and significant than additive gene effect, which itself was nonexistent with this set of switchgrass F1 hybrids. Non-additive genetic variation contributed 5% to the total phenotypic variation in IVDMD. There was no reciprocal effect, neither in the form of nuclear ($GCA \times \text{Reciprocal}$) or non-nuclear ($\text{Cross} \times \text{Reciprocal}$) effect. The remaining contribution originated mostly from environmental influences, in the forms of interactions between GCA with replications and years, and between replications with years, and environmental noise by far. There was no differential performance of the crosses or hybrids across years.

With no additive genetic variation available for in-vitro dry matter digestibility in this experimental switchgrass hybrid population, heritability in the narrow-sense was practically nil. Broad-sense heritability was moderate ($H^2_B = 0.28$ and $H^2_B = 0.20$) at the fullsib-family mean and at the within fullsib-family mean levels, respectively, with contribution derived exclusively from the non-additive component of the genetic variation.

3.2.2. Lignin Content (ADL and KL)

Additive gene action was not responsible for the inheritance of acid detergent lignin (ADL) in this set of switchgrass F1 hybrids, the difference being too small to be statistically significant. The non-additive effect was detected with a 43% contribution to the total phenotypic variation. No reciprocal effects were detected, neither at the GCA or SCA level. Except for some borderline interaction between GCA and years and between SCA and replications, no other interaction was significant. With no additive gene action detected, only broad-sense heritability was estimable, and it was high at both at the fullsib-family mean (0.80 ± 0.35) and within fullsib-family mean (0.73 ± 0.35) levels.

For Klason lignin, non-additive genetic variation was the major source of variation present in the switchgrass population of F1 hybrids, contributing about 24% to the total phenotypic variation. There was some indication of a low level ($VC/SE = 1.02$) of reciprocal effect manifested in an interaction between nuclear and non-nuclear genes ($\text{Cross} \times \text{Reciprocal}$) among the ecotypes. While there was no additive genetic variation among the crosses, there is a possibility ($VC/SE = 1.03$) for the existence of an interaction between GCA and years of testing. The latter two interactions combined accounted for only 9.5% of the total phenotypic variation. Narrow- and broad-sense heritability values for KL were 0.28 ± 0.20 and 0.55 ± 0.15 at the fullsib-family mean level and 0.13 ± 0.11 and 0.49 ± 0.23 at the within fullsib-family mean level.

3.2.3. Ethanol Yield

The analysis revealed significant GCA and SCA effects for ethanol yield contributing 16% and 31% to the total phenotypic variation, respectively. Both additive and non-additive genetic effects were responsible for the inheritance of this trait. No reciprocal effect was detected involving either GCA or SCA. Both GCA and SCA were stable in ETOH performance across years of testing with no interaction detected. However, the interactions between the reciprocal SCA and years of testing and between SCA and replications were significant, indicating a necessity to find genotypes or families with more stable performance across variable years. Narrow- and broad-sense heritability values for ETOH were 0.55 ± 0.21 and 0.82 ± 0.07 at the fullsib family mean level and 0.49 ± 0.13 and 0.73 ± 0.09 at the within-fullsib family mean level.

3.2.4. Relative Importance of GCA and SCA

The Baker's ratios calculated for the five traits ranged from 0.003 to 0.67, with the lowest values (0.003–0.10) being for IVDMD, ADL, and DMY and the highest values (0.51–0.67) associated with KL and ETOH. In this F1 population of inter-ecotypic switchgrass population, non-additive genetic variation was by far more important than additive genetic variation in controlling the inheritance of these five traits.

3.2.5. Combining Ability Effects

Considering the potentials of the parental and reference populations in increasing biomass yield, the only positive SCA effects came from a selection within Kanlow for high cellulose content and high DMY (Table 3). Positive alleles towards increasing IVDMD were contributed by Summer, King's County, and a selection within Summer for late maturity. For reducing lignin content (ADL and KL) for either a forage or bioenergy objectives, King's County and a selection within Kanlow for bioenergy would provide favorable alleles in crosses. To improve ethanol yield, the positive alleles would come from Kanlow, the late-maturing Summer selection, and the bioenergy selection within Kanlow.

Table 3. Specific combining ability effects (BLUP) for the switchgrass populations and their crosses along with % gain of certain crosses relative to the breeding values of the best parent.

| Population or Crosses † | Code ‡ | DMY | Gain (%) § | IVDMD | Gain (%) | ADL | Gain (%) | KL | Gain (%) | ETOH | Gain |
|-------------------------|-----------|-------|------------|-------|----------|-------|----------|--------|----------|-------|------|
| Summer | 1 | −1.79 | | 1.59 | | 0.00 | | 8.11 | | −2.95 | |
| Kanlow | 2 | −0.31 | | −3.98 | | 0.00 | | 7.28 | | 2.68 | |
| King's County | 3 | −1.73 | | 2.10 | | 0.00 | | −11.96 | | −0.72 | |
| Summer (Late maturity) | 4 | −2.10 | | 3.62 | | 0.31 | | 2.50 | | 0.64 | |
| Kanlow (bioenergy) | 5 | 0.95 | | 2.24 | | −0.65 | | −2.79 | | 0.67 | |
| Liberty (cv) | 6 | −0.75 | | −2.49 | | 0.12 | | −1.86 | | −1.14 | |
| KE/KC | 11 × 810 | −0.62 | | 2.55 | | 0.63 | | 4.59 | | 0.38 | |
| KE/KC | 4 × 910 | 1.30 | 0.36 | −5.47 | | 1.24 | | 0.94 | | −0.83 | |
| KE/KC | 8 × 510 | −2.25 | | 5.21 | 0.44 | −0.84 | 0.30 | −6.47 | 1.76 | 0.31 | |
| KE/KC | 20 × 610 | 0.08 | | 4.24 | 0.17 | −0.20 | | −5.30 | 1.27 | 0.25 | |
| KE/KC | 1 × 410 | −1.54 | | −0.55 | | 0.06 | | 0.86 | | −0.33 | |
| KE/KC | 17 × 110 | −0.83 | | −7.12 | | 0.31 | | 2.78 | | −0.56 | |
| KE/KC | 24 × 410 | −1.48 | | 1.46 | | −1.18 | 0.82 | −2.13 | | 0.10 | |
| KE/KC | 7 × 110 | 2.73 | 1.87 | 6.47 | 0.79 | −0.94 | 0.45 | −0.99 | | 3.63 | 0.35 |
| KE/KC | 26 × 10 | −2.65 | | −2.81 | | 1.05 | | 3.11 | | −1.08 | |
| KB/KC | 42 × 10 | −0.51 | | −0.16 | | 0.76 | | 3.71 | | 0.11 | |
| KB/KC | 51 × 910 | −1.04 | | 3.53 | | −0.43 | | −7.13 | −0.03 | −0.84 | |
| KB/KC | 44 × 610 | 1.61 | 0.69 | −0.96 | | 0.12 | | 0.70 | | 1.15 | 0.18 |
| KB/KC | 49 × 810 | 0.80 | −0.16 | 0.11 | | 0.49 | | −0.15 | | −0.84 | |
| KB/KC | 45 × 310 | 3.63 | 2.81 | −0.38 | | 0.32 | | −1.38 | | 1.17 | 0.18 |
| KE/SL | 18 × 618 | −0.92 | | −5.46 | | 1.15 | | 6.37 | | −1.56 | |
| KE/SL | 22 × 611 | 1.14 | | −6.68 | | 1.22 | | 3.55 | | −1.96 | |
| KE/SL | 19 × 616 | 0.08 | | 5.94 | 0.64 | −0.57 | | −3.98 | 3.79 | 0.31 | |
| KE/SL | 12 × 630 | −0.76 | | 0.55 | | −0.43 | | 1.06 | | −0.60 | |
| KE/SL | 2 × 628 | 1.49 | 0.56 | 1.80 | | −0.15 | | 0.83 | | 0.18 | |
| KE/SL | 6 × 633 | 1.21 | 0.27 | 6.01 | 0.66 | −1.81 | 1.80 | −0.62 | | 2.60 | 0.57 |
| KE/SL | 9 × 617 | 0.46 | | −1.45 | | 1.19 | | 2.96 | | −1.48 | |
| KB/SL | 48 × 614 | 2.09 | 1.19 | 2.36 | | −0.92 | | −3.61 | 5.73 | 1.81 | 1.77 |
| KB/SL | 40 × 607 | −3.75 | | 0.23 | | 0.50 | | 3.21 | | −2.17 | |
| KB/SL | 43 × 604 | 1.24 | 0.30 | −2.17 | | 0.04 | | 3.55 | | −0.44 | |
| KC/SL | 310 × 638 | 1.43 | 0.50 | −2.21 | | −1.03 | 0.60 | −8.20 | 0.73 | 1.54 | 0.55 |
| KC/SL | 710 × 601 | 0.72 | −0.25 | 3.33 | −0.08 | −0.45 | | 1.39 | | −0.72 | |
| KC/SL | 710 × 627 | 2.39 | −3.75 | −9.07 | −9.07 | 0.44 | | −0.74 | | 0.95 | |
| KC/SL | 110 × 606 | −0.31 | 3.63 | −2.36 | 6.47 | −0.35 | | −0.19 | | −0.25 | 3.63 |

† KE, a selection within Kanlow (lowland) for early maturity; KB, a selection within Kanlow for bioenergy; SL, a selection within Summer (upland) for late maturity; KC, PI accession of King's County (lowland); Liberty, a cultivar derived from crossing Summer with Kanlow and released for bioenergy (Vogel et al., 2014); ‡ First and second numbers indicate population origin of the parent as per cross-listed on the left column; § Gain (value of cross minus that of best parent divided by best parent value) calculated only for individual crosses showing superiority over the breeding values of the best parent.

Table 3 indicates that not all genotypes from a population with positive SCA will result in crosses that maximize gains for any of the traits. Even the populations which were negative (i.e., SCA) overall for a trait will still include genotypes to provide suitable inter-ecotypic crosses with positive SCA effects or with great potential for substantial genetic gains in the traits. That being the case, careful selection of parents and of outstanding

crosses will have to take place in progeny testing before deploying cultivars with the right combinations of traits for forage and bioenergy.

4. Discussion

4.1. General Assumptions

Disomic segregation is typical in switchgrass even though tetraploidy and octoploidy characterize the two cytotypes used in breeding as found in natural stands [18]. Based on that, there was no concern carrying this type of combining ability analyses with parent plants derived from tetraploid switchgrass populations. Two other issues would be the linkage equilibrium and random mating requirements for such populations to be used in a diallel design [19–21]. Shielded somewhat by the polyploidy background, switchgrass has not been under such a long and intensive breeding history for linkage disequilibrium to be a serious violation of this assumption, and the plants used as parents were randomly selected from these populations, even those that were already under breeding. The matching of plant pairs in crosses was governed exclusively by the synchrony of flowering and not by their performance. Another assumption usually made is that contribution from epistasis is small or absent [21] as the designs do not lend to estimating this effect, even though the absence of epistasis is unlikely in this polyploid background. Homozygosity of the parents is an assumption that cannot be met in switchgrass, being a self-incompatible polyploid and highly heterozygous. However, the interpretation of the causal variance components (as additive, dominance, GCA, SCA, etc.) is reasonable as the main goal of this study was to understand the interaction of these three populations/genomes in crosses, extract the magnitude of the variance components, and explore new breeding strategies by applying a statistical model with pedigree information, that is expected to control over- or under-estimation of the genetic components [13,19].

4.2. Implications for the Breeding Program

The analyses of this switchgrass population clearly indicated that nonadditive genetic variation played a significant and predominant role in the inheritance of the five traits considered in this study. There is compelling evidence that dominance and/or epistasis matter to a great extent when inter-ecotypic crosses are exploited in breeding switchgrass. Dry matter and ethanol yields were the only two traits for which both GCA and SCA were important, in the form of a reciprocal GCA effect for DMY, indicating that breeding values of parents can predict progeny performance of DMY and ETOH in crosses. The northern lowland ecotypes, such as cv Kanlow, have higher biomass yield and disease resistance than the northern upland ecotypes, such as cv Summer, which are more frost-tolerant, earlier maturing, and, for so, less yielding in the Nebraska environments [5,6]. To explain the significant reciprocal effect for DMY, it is plausible that, to design crosses between these two ecotypes (i.e., upland-lowland) for the NE environments, the lowland cytoplasm/mitochondria may be more tailored as female to producing higher biomass than that of the upland ecotypes. There is evidence that also applies to intra-population breeding in switchgrass since [22] reported site-independent reciprocal effects for biomass yield in lowland switchgrass.

Except for DMY, the reciprocal effect did not seem to be important for the other four traits. However, there were indications of interactions between the nuclear and cytoplasmic genes (Cross \times Reciprocal) when KL is a target and those interactions combined with year were significant for ETOH. Such interactions usually result in a negative correlation between KL and ETOH in bioenergy breeding of switchgrass for the lignocellulosic platform, with genotypes having less KL being the ones producing more ethanol [6].

With GCA almost absent in this population of switchgrass hybrids, heritability values were expressed at the fullsib family level. These heritability values ranged from moderate to very high and can be used in a combined selection index to select families and individuals within families and maximize genetic gains. Ample GCA or additive variation is usually found in genetic studies of intra-population breeding to make selection and progress in

switchgrass [5,7]. Therefore, it is difficult to completely rule GCA out as an important source of variation to the traits since the design used the parent plants in only one cross. Using clones of the same parent plants in two to three crosses at least with two to three different genotypes might have improved the precision of highlighting the importance of GCA.

It is not possible to generalize the findings of this study to the whole breeding program, which is mainly conducted at the intra-population level. The interactions between nuclear genes and between nuclear and non-nuclear genes, which cause the non-additive variation with these inter-ecotypic crosses, were to be expected, as these ecotypes have explored different selection pressures from geography, climate (drought, freeze), insects, and pathogens that impacted their phenology, survival, and growth potential [23–25]. This germplasm is to be seen as a source of different and potentially superior alleles to exploit for complementary gene action. An introgression breeding may be justified here to impart, for example, freeze (overwintering) tolerance in lowland or disease tolerance in upland breeding populations while improving biomass yield and quality traits. The allelic interactions expressed as SCA effects across traits can be exploited to increase biomass yield further than it is possible with relying on GCA alone.

The decision was made in this breeding program to augment the breeding populations with some biparental crosses using parents having the highest breeding values, particularly for biomass. Nonetheless, dominance variation cannot be fixed or inherited from parents to progeny, as compared with additive genetic variation, as it breaks down with recombination [26]. Vogel and Mitchell [27] reported a loss of the initial heterosis found in switchgrass due to recombination across generations. The benefits from this strategy can be extended to the production population whereby specific families can be selected for release or clonal hybrids can be deployed, pending the availability of a high-throughput protocol to develop clones from switchgrass [8,28]. The gains from selection experiments would be immediately captured by farmers as selection is practiced on the total genetic variation (additive + dominance + epistasis), as it is conducted in the breeding of other vegetative crops, such as sugarcane (*Saccharum spp* hybrids). That would require a total paradigm shift in switchgrass production for bioenergy.

5. Conclusions

Non-additive genetic variation was the primary and predominant source of variation detected in this study of inter-ecotypic crosses involving lowland, upland, and advanced switchgrass breeding lines. Selection and deployment of fullsib families with selection between and then within families in a combined index would be necessary to capture the selection gains possible with this strategy. A salient result from this research is that an additional reciprocal GCA effect was detected for biomass yield, suggesting the use of the highest biomass parent as female in inter-ecotypic crosses when bioenergy is the goal. At this stage of switchgrass breeding programs, it appears that introgression with new materials into a breeding population can be done whenever possible to increase the effective population size (diversity) and create superior progeny from crosses of an inter-ecotypic nature. That would assume a careful selection and crossing of parents with the highest breeding values via progeny testing. Cloning for rapid deployment will be necessary to immediately capture potential gains from non-additive (dominance) genetic variation in the context of switchgrass breeding, bypassing the additional steps of recombination.

Author Contributions: S.E., conceptualization, methodology, analysis, research, and writeup; R.M., research and review of manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by USDA-ARS CRIS Project # 3042-21000-034-00D.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No data is being made available for now.

Acknowledgments: The authors are grateful to Ken Vogel for planning/implementing the research in the years 2011–12; Steve Masterson for NIRS predictions of field data; and to Patrick Callahan for field activities.

Conflicts of Interest: The authors declare no conflict of interest.

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