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RESEARCH ARTICLE

Biomass yield improvement in switchgrass through genomic prediction of flowering time

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Abstract

The seasonal timing of the transition from vegetative to reproductive growth has a major impact on biomass accumulation in switchgrass. Late-flowering switchgrass cultivars produce greater biomass, a critical trait for sustainable bioenergy production. Genomic prediction (GP) may allow rapid selection of late-flowering individuals with reduced time and expense for field evaluations. To evaluate GP, two flowering time traits (heading date and anthesis date) were collected on 1532 genotypes from four breeding populations: Midwest, Gulf, Atlantic, and Hybrid. These were sequenced using genotype-by-sequencing (530,792 single-nucleotide polymorphisms). Predictive ability of single-trait and multi-trait models were evaluated by cross-validation, by prediction of a progeny trial ($n = 122$), and through prediction of yield performance in a parallel experiment ($n = 52$). Predictive ability was not improved by sharing information among breeding groups. Overall, multi-trait models provided an advantage during cross-validation, but a smaller advantage during progeny prediction. Within populations, GP resulted in lower per-cycle progress than previously reported field evaluations (3.1 vs. 5.0 day^{-1} cycle^{-1}). However, GP cycles are potentially much faster than field evaluations. When directly predicting biomass yield, the Hybrid training population had a predictive ability of 0.54–0.63. This reinforces the strong linkage between biomass yields in swards and flowering time. These results highlight the value of GP for rapid yield improvement in switchgrass, particularly in a breeding program designed to share information between biomass yield trials and low-cost flowering time evaluations.

KEYWORDS

climate change, crop yield, flowering time, genomic prediction, grass breeding, switchgrass

1 | INTRODUCTION

Energy cropping systems are a potential replacement for petroleum energy sources which are inducing global climate change (Aravindhakshan et al., 2010; Gelfand et al., 2020). However, energy cropping systems must overcome multiple hurdles: the industry lacks infrastructure, must contend with already high land demand (based on both agricultural or conservation value), and must domesticate entirely novel high-energy yielding crops. The perennial grass switchgrass (*Panicum virgatum* L.) is a potential biomass energy source or feedstock for high-value fuels or products (Masum et al., 2020; Sanderson et al., 2006; Wright & Turhollow, 2010).

Switchgrass contains three major ecotypes and many regional subpopulations (Evans et al., 2018; Lovell et al., 2021). Midwest ecotypes generally have greater cold and drought tolerance and naturally occur in the northern continental United States. Gulf ecotypes occur in the southern United States and Mexico and tend to produce greater biomass, have greater diversity, and have greater disease resistance (Uppalapati et al., 2013; VanWallendael et al., 2020; Zalapa et al., 2011). Atlantic ecotypes occur along the Eastern United States and contain a range of flowering times and winter survival traits (Lovell et al., 2021). Hybrid cultivars of Midwest and Gulf populations have been created in an effort to combine beneficial traits from distant genetic pools (Casler & Vogel, 2014). Determining which switchgrass germplasm and breeding strategies will provide the greatest long-term yield gain for the north-central United States is an unresolved question (Casler, Vogel, et al., 2018).

Biomass yield is the primary economic limitation for sustainable switchgrass production (Brandes et al., 2018; Fike et al., 2017; Perrin et al., 2008). Breeders have reported prior yield gains between 1%–4% per selection cycle, for selection cycles that generally require 4 to 6 years (Casler & Vogel, 2014). More rapid yield progress is limited by long cycles of selection based on direct measures of phenotype, by low heritability for sward yield (Bhandari et al., 2011; Nayak et al., 2020; Ramstein et al., 2016), and by resource-intensive measurements (Casler, 2020; Casler & Ramstein, 2018). One alternative strategy for yield improvement in switchgrass is selection for delayed flowering (Casler, 2019; Casler, Vogel, et al., 2018). Individuals selected for delayed flowering time produce greater yield in many crops harvested for biomass due to an extension of vegetative growth and greater utilization of the growing season (Cuevas et al., 2016; Jensen et al., 2011; Schwartz & Amasino, 2013). Delayed flowering also results in an increase in height, which is an additional surrogate for yield (Boe & Casler, 2005; Van Esbroeck et al., 1998). However, height is subject to increased environmental variance relative to flowering time (Bhandari et al., 2011; Dalid et al., 2018; Price & Casler, 2014a, 2014b, 2014c; Vogel & Mitchell, 2008).

Morphologically, floral development in switchgrass begins with panicle emergence from the leaf sheath (heading), continues through anthesis, fertilization, and eventual seed ripening (Moore et al., 1991). Exact dates for fertilization and seed ripening are difficult to identify, but the date of initiation of heading and anthesis can be visually determined and recorded. High correlations between anthesis and heading date have been reported in switchgrass ($r > 0.9$; Lipka et al., 2014; Price & Casler, 2014a; Taylor et al., 2018). The major advantages of flowering time selection are its high heritability and strong relationship as a surrogate for sward yield (Casler & Ramstein, 2018; Price & Casler, 2014a). The relationship between delayed flowering time and yield is well studied in the Midwest ecotype, which flowers and begins to senesce early in the growing season (Casler, 2019). The impact of delayed flowering time on yield has not been reported for hybrid breeding populations and Gulf ecotype cultivars. An experiment by Van Esbroeck et al. (1998) carried out a single cycle of divergent selection in the Gulf cultivar Alamo. While yield was not recorded, late-flowering plants produced more leaves per tiller and were taller than early flowering selections in Texas. In Wisconsin, two cycles of selection within a northern adapted Midwest population resulted in a 11-day difference between early and late selections, which corresponded to a yield difference of 2.0 Mg ha^{-1} (Price & Casler, 2014a). In a subsequent experiment, divergent selection for flowering time was carried out on two northern adapted populations and reported an average gain of 5 days cycle^{-1} with an associated average yield increase of 0.54 (2.3%) Mg ha^{-1} cycle^{-1} (Casler, 2019).

Genome-wide prediction (GP) simultaneously assigns marker effects to a dense array of genetic markers to predict breeding values for genotypes in a population. GP outperforms prior prediction methods for quantitative traits (Arruda et al., 2016; Crossa et al., 2017). For switchgrass, adoption of GP can accelerate breeding progress by improving selection accuracy for low-heritability traits, reducing the need for phenotyping, and accelerating selection cycles (Casler & Ramstein, 2018; Lipka et al., 2014; Poudel et al., 2019). With GP, a selection cycle can occur in a single season, a reduction from up to 5 years per cycle for biomass yield (Simeão Resende et al., 2014). Promising GP accuracies have been reported for many switchgrass traits, although many of these prediction accuracies are inflated due to population structure (Fiedler et al., 2018; Lipka et al., 2014; Poudel et al., 2019; Ramstein & Casler, 2019; Ramstein et al., 2016). Prior GP accuracy within breeding populations for yield have been 0.20–0.23 (Casler & Ramstein, 2018; seeded sward plot yield) and 0.10–0.25 (Fiedler et al., 2018; spaced plant plot yield). While progress can be made with low GP accuracy, these values result in marginal overall gains relative to phenotypic selection for yield and any erosion of prediction accuracy will result in negligible improvements (Casler & Ramstein, 2018).

An alternative is to apply GP to flowering time as a yield surrogate (Casler & Brummer, 2008; Casler & Ramstein, 2018). Compared to GP of yield, GP of delayed flowering time has multiple advantages. Flowering time can be reliably measured in unreplicated spaced-plant conditions and yield improvements due to delayed flowering time can improve performance across a range of environments (Casler, 2019). The aim of this study is to evaluate GP as a strategy to select for delayed floral initiation in switchgrass breeding populations. Specifically, this study will (1) quantify the repeatability and predictive ability for flowering time traits in various switchgrass breeding populations (2) evaluate the utility of sharing genomic information between switchgrass breeding populations, (3) determine the effect of multi-trait (MT) models on GP, and (4) validate-flowering time predictions using cross-validation, a progeny trial, and a sward yield trial.

2 | MATERIALS AND METHODS

2.1 | Field experimental design

In this study, the experimental units for the GP training data were unreplicated, randomized greenhouse-grown spaced plants (previously described in Tilhou & Casler, 2021). The training population nursery was established in row plots in 2016 at a single location (Arlington, WI; 43° 18′ N; 89° 21′ W). Each row contained seedlings from a single breeding population with 90 cm between rows, 30 cm within rows and 25 plants per row. To simplify data collection, alternating seedlings were killed in spring 2018, resulting in up to 13 plants per row spaced 60 cm apart ($n = 1532$). The training population was blocked into sets of 20 rows within the Midwest and Hybrid breeding groups. Gulf ecotype seedlings were planted across four plots within the same field and each plot was considered a block. Due to winter mortality, blocks consisted of between 80 and 128 unreplicated seedlings.

Training populations included Midwest ecotypes, Gulf ecotypes derived from Kanlow, a stabilized hybrid cultivar (Liberty), and a diverse set of recently collected populations including both Gulf and Atlantic ecotypes. All genotypes are tetraploids undergoing selection for cold tolerance and late-flowering traits. The Midwest population is the results of one cycle of selection for late-flowering and greater height from the WS4U population (Casler et al., 2006). The Hybrid populations is from a stabilized cultivar from Midwest (Summer) and Gulf (Kanlow) parents (Vogel et al., 2014).

A row-plot validation trial was established using seed collected from open-pollinated plants selected from the training nursery. Due to uneven germination, the validation nursery only included families collected from Hybrid (5 families) and Midwest parents (5 families). These seedlings were randomly assigned to family rows (15 seedlings per row) and rows were assigned using a completely randomized design (30 cm between plants, 90 cm between rows; $n = 28$–45 progeny per family). The validation trial was planted in May 2020 at the same location as the training study. All plants were allowed to grow during the establishment year and biomass was removed after killing frost. No fertilizer was applied during the establishment year. Genotypes within this trial were used to validate GP.

2.2 | Phenotypic data collection

Heading date was recorded as the day of year with 50% of panicles being visible. Anthesis date was recorded as the day of year in which at least 50% of panicles contain 50% protruding anthers (Casler & Ramstein, 2018). In 2018, only anthesis data was collected on the training populations. During 2019 and 2020, heading and anthesis dates were collected on the training populations. During the summer of 2021, heading and anthesis dates were collected on the validation population. Measurements from 2018, the first year of data collection, were discarded if they were determined to be biologically implausible and were attributed to human error during the first year of data collection. Specifically, anthesis dates measurements which differed by greater than 2 weeks from mean of anthesis dates recorded during 2019 and 2020 were set as missing.

2.3 | Generation of best linear unbiased predictions

One- and two-stage linear mixed models were used to generate best linear unbiased predictors (BLUPs) for genotypes (Cullis et al., 1996; Schulz-Streeck et al., 2013). The following models were also used for trait prediction by masking the observed performance of genotypes and generating genomically estimated breeding values (GEBVs). The correlation between GEBVs and BLUPs is the predictive ability for the model. The following single-stage linear model was used to obtain BLUPs of flowering time (anthesis date, heading date) using either the complete training dataset or subsets containing different breeding populations:

$$y_{jkl} = \mu + p_j + M_k + b_l + \epsilon_{jkl},$$

where y_{jkl} is an observation, p_j, M_k and b_l are the effects of an j-th genotype (random) in a k-th year (fixed) and l-th block (fixed), and ϵ_{jk} are residuals (i.i.d. ~$N[0; \sigma_\epsilon^2]$). The symbol μ represents the experiment-wide mean. Genotypic effect variance–covariance structures were estimated using marker relationships ($p_j \sim \mathrm{MVN}[0, \mathbf{G}\sigma_g^2]$). The **G** matrix of genomic relationships between genotypes was calculated using A.mat in the rrBLUP package (Endelman, 2011). This model was solved using the R package "sommer" (R Core Team; Covarrubias-Pazaran, 2016).

Because of uneven measurement effort between the flowering time data collection (2 years for heading date and 3 years for anthesis date), MT models used a two-stage approach where adjusted means were calculated for each trait individually, then MT models were used to obtain genomic BLUPs. For the second stage, adjusted means were weighted based on the inverse of the square root of their standard error from the first stage (Cullis et al., 1996; Schulz-Streeck et al., 2013). For the first stage of analysis, adjusted means were obtained using the model:

$$y_{jkl} = \mu + p_j + M_k + b_l + \epsilon_{jkl},$$

where y_{jk} is a yield surrogate, p_j, M_k and b_l are the effects of an j-th genotype (fixed), k-th year (fixed), and l-th block (fixed), and ϵ_{jk} are residuals (i.i.d. ~$N(0; \sigma^2_\epsilon)$).

For the second stage, the adjusted means were used to predict trait BLUPs using the following model:

$$y_{jk} = \mu + g_j + \epsilon_{jk},$$

where y_{jk} is adjusted means of the observed trait, μ is the overall mean, g_j, is the effect of the j-th genotype and ϵ_{jkl} are residuals. For the MT model, y_{jk} consisted of anthesis and heading date BLUEs, which were predicted simultaneously assuming unstructured covariance between traits. Genotypic effect variance–covariance structures were estimated using marker relationships (g_j ~ MVN[0, $\mathbf{G}\sigma^2_g$]).

Repeatability was calculated as

$$R^2 = v_\Delta^{\mathrm{BLUP}} / 2\sigma^2_G,$$

where σ^2_G is genotypic variance, and v_Δ^{BLUP} is the mean variance between all pairs of genotypic BLUPs (Cullis et al., 2006; Schmidt, Hartung, Rath, et al., 2019). This alternative heritability estimator can more accurately account for unbalanced experimental designs and resulted in more realistic repeatability estimates in populations containing population structure.

For the validation genotypes from a companion experiment (SPACE, described below), reliability was calculated as

$$r_i^2 = 1 - \frac{Vg_i^{\mathrm{BLUP}}}{Vg_i},$$

where Vg_i^{BLUP} is the prediction error variance for the genotype i (from the diagonal of the $\mathbf{C}_{22\mathrm{g}}$ matrix), and Vg_i is the diagonal of the $\mathbf{G}$ matrix (Schmidt, Hartung, Bennewitz, et al., 2019). This statistic is similar to heritability and is calculated on a genotype-difference basis, providing individual estimates for genotypes (Schmidt, Hartung, Bennewitz, et al., 2019).

2.4 | DNA sequencing and analysis

Leaf samples were collected for DNA extraction and genotyping by sequencing during the fall of 2020. Genotype-by-sequencing occurred on an Illumina NovaSeq 6000 (2 × 150 bp) sequencer at the University of Wisconsin Biotechnology Center, targeting a read depth of 250 million reads per sample. The data analysis of sequencer outputs was performed in TASSEL (Glaubitz et al., 2014). Sequencer outputs were aligned to the reference genome (*P. virgatum* v 5.1) to call single-nucleotide polymorphism markers (SNPs). Resulting SNPs were filtered for minimum taxa coverage (0.01), minimum locus coverage (0.2), minor allele frequency (0.05), and for significant linkage disequilibrium with at least one other marker. Imputation of missing markers (37%) was done through the Beagle program, resulting in a total of 530,810 markers (Browning et al., 2018). Individual ancestry was evaluated using the R package LEA (Frichot & François, 2015), which calculates admixture coefficients for each genotype using sparse non-negative matrix factorization, a strategy similar to STRUCTURE (Pritchard et al., 2000). Principal components of marker data were calculated to evaluate the degree of population structure within and between the breeding groups based on markers in linkage equilibrium ($r < 0.2$; 97,901 markers). Weir and Cochran's weighted F_{st} was also calculated between breeding groups with potential admixed genotypes (max LEA admixture coefficient <0.5) omitted (Weir & Cockerham, 1984). For GP, markers were pruned based on linkage disequilibrium with pairwise correlations >0.95, reducing the number of markers to 430,792.

2.5 | Prediction validation strategies

Broadly, the predictive ability of the above models was evaluated using leave-one-out cross-validation, progeny validation, and by yield prediction in a parallel experiment. Leave-one-out cross-validation involves masking the observations of a single genotype from a model and generating a GEBV for the individual. This process is then repeated for all individuals in the target population and the correlation between GEBVs and BLUPs is calculated as the predictive ability. Leave-one-out cross-validation was used within each breeding group in the training population to assess predictive ability both with and without supplemental information from additional breeding groups. Leave-one-out cross-validation was also used to evaluate ST and MT models within each breeding group. When validating ST and MT models, ‘true’ breeding values were assumed to be those predicted within the ST and MT models, respectively.

Validation was also carried out on individuals from half-sib families derived from parents selected from the training population (validation trial described above, $n = 122$) by predicting progeny BLUPs using GEBVs derived from the training dataset. Finally, the model was applied to a field trial of 52 half-sib families of Gulf ($n = 20$) or Hybrid ancestry ($n = 32$), referred to as the SPACE study (Tilhou et al., 2022). Families in this field trial were evaluated for multiple traits, including sward yield (2.5 observations per family) for 2 years. The merged marker arrays from the current study and SPACE study resulted in 48,265 shared SNPs. The combined dataset will allow quantification of the relationship between flowering time and sward yield in Gulf and Hybrid populations.

3 | RESULTS

Principal component analysis largely aligned with expectations for the Midwest and Hybrid breeding groups (Figure 1). The Gulf nursery contained multiple subpopulations and a large degree of diversity. Based on admixture coefficients, the Gulf population was subdivided into two breeding groups (Figure S1; Table S1). These groups were assigned to Gulf and Atlantic breeding groups with the latter group conforming with reports of germplasm from the United States East Coast (Lovell et al., 2021). These Atlantic subpopulations were recently incorporated into the nursery and have not cross-bred with the primary Gulf breeding group or each other. Therefore, multiple clusters can be observed within the Atlantic breeding group (Figure 1). By contrast, the Hybrid and Midwest breeding groups resulted in single clusters, with minimal evidence of within-breeding group population structure (Figure 1). Breeding group identity had a large influence on the mean and variance of flowering time traits (Figure 2).

An initial cycle of leave-one-out validation for anthesis date resulted in minor or major reductions in predictive ability when unrelated breeding groups were included in the training population for predictions (Table 1). This was further supported by a low (0.02) mean pair-wise correlation between breeding group marker effects for anthesis date (not presented). The only exception was an improvement in predictive ability in the Atlantic breeding group when predicted using the complete dataset. This represents improved accuracy due to similarity among individuals within subpopulations, which will not be useful for a random mating population. This strong population structure within the Atlantic breeding group makes it unrepresentative of a realistic breeding population. Therefore, the Atlantic subpopulations were omitted from remainder of the analysis.

Within the remaining breeding groups, repeatability estimates for flowering time traits were moderate to high in both the ST and MT models (Table 2). The genetic correlation between the two flowering time traits were strong in MT models (Table 2). The Midwest breeding group contained the lowest additive genetic variance for both traits (12.3 and 3.7 for anthesis and heading date, respectively) but the highest repeatability for both flowering time traits

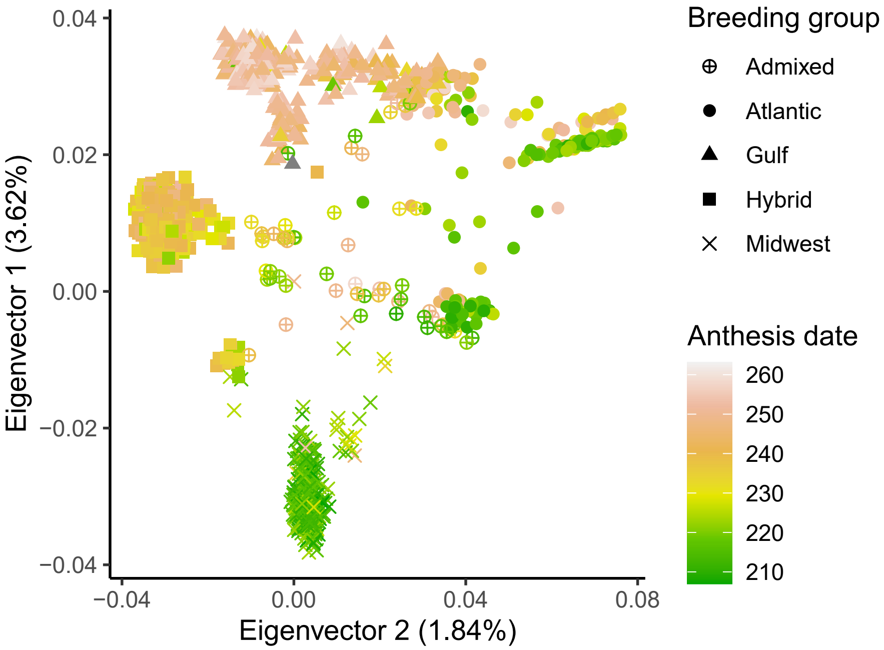

FIGURE 1 The first two principal components of 1532 switchgrass genotypes based on 97,901 single-nucleotide polymorphism markers in linkage equilibrium ($r^2 < 0.2$). Point shapes indicate breeding group identity and color indicates the mean observed anthesis dates. The percent variance of each principal component are indicated in parentheses.

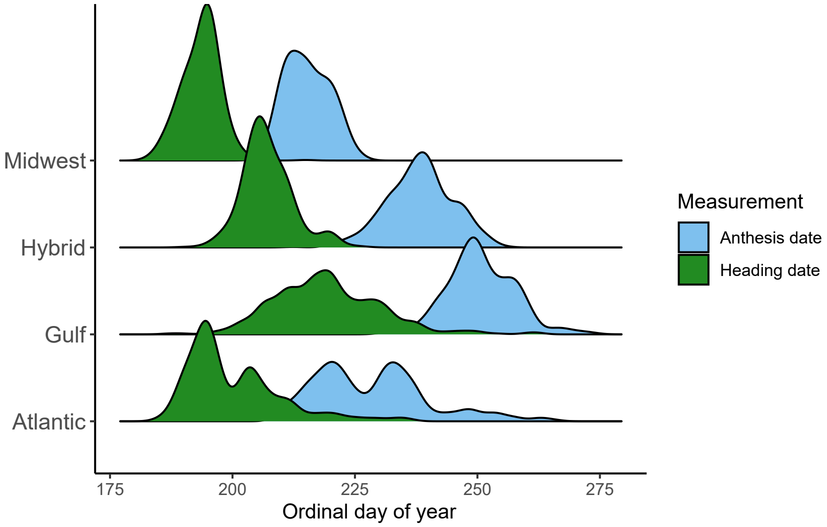

FIGURE 2 A histogram of the mean heading dates and anthesis dates (ordinal day of year) of genotypes in four breeding groups. The histogram is normalized for sample size due to differences in breeding group population size. The area under each curve is equal to one.

TABLE 1 Genomic prediction ability for anthesis date within and between switchgrass breeding groups. Each scenario used leave-one-out validation where training datasets were composed of the remainder of the target population and the supplementary group. The complete supplementary training scenarios incorporated genotypes from all breeding groups. Diagonal values (bold) indicate predictive ability without supplementary training populations

Supplementary training dataset	Target population			
	Gulf	Atlantic	Midwest	Hybrid
Gulf	**0.70**	0.60	0.55	0.41
Atlantic	0.18	**0.60**	0.44	0.34
Midwest	0.69	0.54	**0.56**	0.33
Hybrid	0.67	0.58	0.52	**0.43**
Complete	0.53	0.68	0.51	0.38

(0.8 and 0.75 for anthesis and heading date, respectively). The MT model resulted in an increase in repeatability for every breeding group-trait combination, with the exception of Gulf anthesis (0.77 to 0.64, Table 1). Genotypic correlations between flowering dates were approximately one for both Gulf and Hybrid breeding populations and 0.77 for the Midwest breeding population.

Predictive ability based on leave-one-out cross validation was greater in the Gulf breeding group relative to Midwest and Hybrid groups for both flowering traits (Table 3). The Gulf breeding group also had greater MT cross validation predictive ability relative to other breeding groups, despite having a reduced repeatability estimate in MT models (Table 2). The MT model resulted in increased predictive ability for all breeding group-trait combinations with the exception of heading date prediction in the Midwest breeding group (0.65 to 0.59, Table 3). Progeny predictive ability was lower than cross-validation estimates for the Midwest breeding population, but equal to or greater in the Hybrid breeding population. Relative to cross-validation, MT models provided similar improvement over to single-trait models within the progeny validation with the exception of heading date in the Midwest breeding group. Predictions of sward biomass yield within the Gulf and Hybrid breeding groups ranged from low to strong. Overall, prediction of Gulf sward yield was nearly zero, with insignificant ($p > 0.05$) relationships between predicted flowering time and yield. Flowering time predictive ability for yield was surprisingly strong within the Hybrid breeding population (0.30–0.43; Table 3). Heading date predictions resulted in greater predictive ability relative to anthesis date and MT models also improved predictive ability relative to ST. Within the 17 Hybrid families with GEBV reliability estimates greater than zero, predictive ability ranged from 0.54 to 0.63.

Within the flowering time measurements, there was minimal inter-annual variation. Therefore, correlations among BLUPs from subsets of the training data simulating reduced field measurement effort indicated generally strong correlations between subset BLUPs and the entire dataset (Table S2). This indicates that 1 year of measurements can often be sufficient for ranking individuals in a breeding population.

4 | DISCUSSION

4.1 | Sharing information among breeding groups was not valuable

This study incorporated a wide array of switchgrass ecotypes and breeding populations. Despite the use of isolated crossing blocks for breeding populations, a number of admixed or intermediate genotypes were

TABLE 2 Mean flowering date (ordinal day of year), additive genetic variance (σ_A^2), error variance (σ_e^2), repeatability (R^2), and genetic correlation (r_G) for both heading date and anthesis date across three switchgrass breeding groups and two modeling strategies, single-trait (ST) and multi-trait (MT)

		Anthesis date				
Breeding group	**Mean**	**ST σ_A^2**	**ST σ_e^2**	**ST R_2**	**MT R^2**	**MT r_G**
Gulf	250	14.1	2.6	0.77 ±0.03	0.64 ±0.04	1.05
Midwest	215	12.0	8.6	0.80 ±0.02	1.00 ±0.04	0.77
Hybrid	237	17.5	39.8	0.56 ±0.03	0.72 ±0.03	0.97
		Heading date				
Breeding group	**Mean**	**ST σ_A^2**	**ST σ_e^2**	**ST R_2**	**MT R^2**	**MT r_G**
Gulf	218	22.3	32.4	0.67 ±0.03	0.69 ±0.03	1.05
Midwest	194	3.6	4.6	0.75 ±0.02	1.00 ±0.03	0.77
Hybrid	206	6.0	12.2	0.63 ±0.03	0.78 ±0.03	0.97

TABLE 3 The predictive ability of a training population (breeding group) based on single trait (ST) and multi-trait (MT) genomic models for flowering time using cross validation, progeny validation, and validation via sward yield prediction

	Anthesis date predictive ability					
	Leave one out cross validation		**Progeny validation**		**Yield validation**	
Breeding group	**ST**	**MT**	**ST**	**MT**	**ST**	**MT**
Gulf (n = 292)	0.70	0.74	—	—	NS/NS[a]	NS/NS[a]
Midwest (n = 529)	0.56	0.69	0.50	0.61	—	—
Hybrid (n = 420)	0.43	0.69	0.73	0.90	NS/0.54[a]	0.30/0.58[a]
	Heading date predictive ability					
	Leave one out cross validation		**Progeny validation**		**Yield validation**	
Breeding group	**ST**	**MT**	**ST**	**MT**	**ST**	**MT**
Gulf (n = 292)	0.71	0.74	—	—	NS/NS[a]	NS/NS[a]
Midwest (n = 529)	0.65	0.59	0.60	0.60	—	—
Hybrid (n = 420)	0.63	0.68	0.63	0.81	0.38/0.60[a]	0.43/0.63[a]

Note: NS, not significant ($p < 0.05$).

[a] Yield predictive abilities are presented for the complete dataset (n = 20 and n = 32 for Gulf and Hybrid families, respectively) and for predictions with repeatability greater than zero (n = 6 and n = 17 for Gulf and Hybrid families, respectively).

found (Figure 1). This could be due to seed contamination, mixed parental ancestry, or accidental genotype replacement. Particularly among breeding populations with Gulf ancestry, winter damage can allow a volunteer seedling to overtake the planted seedling. The Atlantic breeding population was also often difficult to differentiate from Gulf individuals and contained a high level of genetic structure and variable flowering time and winter survival traits (not presented). These new populations and admixed individuals will require further evaluation to determine their value in a long-term breeding program. This inadvertent survey of breeding population diversity highlights the challenge of maintaining seed purity within a breeding program of outcrossing semi-wild crops.

In this experiment, the Gulf breeding group had a number of unexpected results, such as predictive ability greater than repeatability, a reduction in repeatability in MT models, a genetic correlation greater than 1, and poor performance in yield prediction validation. These anomalies are most likely the result of population structure. Alternatively, there could be a confounding association between winter injury and flowering time (not presented). The Gulf population contained individuals which suffered from chronic winter damage which could delay flowering time measurements due to delayed spring recovery and growth.

In agreement with prior reports, supplementing TPs with distant relatives of the target population did not improve predictive ability (Table 1; Desta & Ortiz, 2014; Heffner et al., 2011; Riedelsheimer et al., 2013). A reasonable expectation of shared linkage blocks between genotypes is required for accurate predictions (Gianola, 2021; Ramstein & Casler, 2019). The exception to this trend was an improvement in predictive ability within the heterogeneous Atlantic breeding population (Table 1). This can be attributed to more accurate prediction of sub-population identity for a genotype, rather than more accurate predictions of genotype performance within sub-populations. Broad TPs therefore could be useful for predicting traits of a genotype of unknown or admixed origin, but this training data will provide no benefit within an organized, recurrent selection breeding program.

4.2 | Potential progress in flowering time and yield through GP

The training populations used in this experiment could predict flowering time with accuracy sufficient for gain equivalent to or greater than phenotypic selection for flowering time. Further, these gains could be maintained with substantial reductions in phenotyping effort (Tilhou & Casler, 2021; Table S2). Assuming selection of the top 10% of a population (selection intensity: 1.75), the repeatability, variance, and predictive ability estimates from progeny validation indicate that Midwest and Hybrid breeding groups could delay anthesis by 3.4 (MT 3.7) and 7.1 (MT 7.8) days in one selection cycle, respectively. For the Midwest population, this is slightly below the realized gains of 5 days per cycle for phenotypic selection reported by (Casler, 2019). In extrapolating this to yield, based on either Casler (2019; 0.13 Mg ha^{-1} day^{-1}) or Price and Casler (2014a; 0.18 Mg ha^{-1} day^{-1}), the above flowering time alterations would increase yields from 0.44–0.67 Mg ha^{-1} cycle^{-1} for the Midwest breeding group. Again, this value is slightly slower than a 2-year visual selection regime for biomass on a per-cycle basis (Casler & Vogel, 2014). However, GP cycles only require a single year, compared to the 2 years required for surrogate field measurements (or longer for yield measurements). Notably, a rapid GP cycle could also occur over winter (Alexander et al., 2014; Castro et al., 2011). The rate of predictive ability erosion is unknown for a switchgrass breeding program with recurrent selection for which there is no updated training population. However, this erosion of predictive ability would have to be substantial to result in lower predicted progress compared to phenotypic evaluations.

The relationship between flowering time and yield has only been reported for breeding populations derived from early flowering Midwest ecotypes. This experiment found evidence of a strong relationship between sward yield BLUPs and flowering time BLUPs in Hybrid populations, with a slope of 0.43 Mg ha^{-1} day^{-1} in 17 Hybrid populations (Casler, 2019; Price & Casler, 2014a). The Hybrid population correlations between flowering time BLUPs and seeded sward BLUPs were comparable to the yield surrogates evaluated in the SPACE study (Tilhou & Casler, 2022). For example, SPACE genetic correlations between seeded sward yields and alternate breeding plot types (using spaced plants rather than seeded swards) ranged from 0.52–63. The flowering time BLUP predictions were also comprable than the internal reliability measure for seeded swards in the SPACE experiment (mean $r^2 = 0.48$). Lastly, the predictive ability for sward yield BLUPs using flowering time was 0.54–0.63 within reliable families, which is greater than previously reported GP accuracy for sward yield of 0.20–0.23 (Casler & Ramstein, 2018). Even accounting for the small sample size of the SPACE experiment, this relationship indicates that flowering time and yield may be strongly linked and can account for a major portion of variance for sward yield observed in the SPACE study.

The MT models evaluated in this study could shorten the time required for genotype evaluations by modelling anthesis date and heading date as correlated traits. Specifically, these MT models allow multiple measures in a single growing season. Generally, MT models are the most effective when one easily measured trait correlates with an important low-heritability or difficult to measure trait (Bhatta et al., 2020; Lado et al., 2018; Lenz et al., 2020). In the current study, there was a modest benefit in cross validation predictive ability despite combining two easily measured traits (Table 3). Although the MT improvement resulted in only marginal improvements in the progeny and yield validation strategies, MT appears to improve single-year BLUP estimates, particularly for anthesis date (Table 3; Table S2). Collecting only a single year of field data allows a breeder to update training populations more rapidly. A further, low-cost addition to a single-season MT strategy would be plant height, which appears to have lower heritability, but is linked to both flowering time and biomass yield (Bhandari et al., 2011; Casler & Ramstein, 2018; Nayak et al., 2020; Price & Casler, 2014a).

4.3 | Breeding opportunities going forward

Overall, this experiment indicates that rapid recurrent GP for flowering time could provide more rapid progress per year than phenotypic selection for sward yield. However, selection based exclusively on flowering time is not the optimum strategy. Simeão Resende et al. (2014) concluded that GP is the most beneficial when any of the following are true: (a) individual measurements for a trait is impossible, (b) a breeder cannot select within families, or (c) measurements are time intensive. GP for flowering time meets none of these criteria. In addition, recurrent GP for flowering time alone could erode the variance in a population or decouple flowering time from other mechanisms for increased yields (Casler, 2019).

Therefore, flowering time measurements are the most useful as a supplement to yield evaluations. Casler and Ramstein (2018) reported that supplementing selection for yield with an additional yield surrogate resulted in 10%–32% greater progress using among and within family selection (Casler & Brummer, 2008). The advantage of among and within family selection is that selection pressure is placed among families on biomass yield, using sward plots, and within families on flowering time using spaced plantings. This also becomes a drawback to among and within family selection, because information is not shared between the yield measurements of half-sib families and individuals measured for surrogate traits. This results in a large amount of wasted labor. Using a MT model, a breeding program could instead incorporate parallel yield and surrogate trait measurements. Specifically, a breeder could collect both flowering time and yield from half-sib sward plots but only flowering time from a larger set of genotypes planted in spaced plant nurseries. By modelling the genetic correlations between flowering time and yield, this strategy could provide yield predictions for a large number of spaced plants in the training population where only the flowering time has been recorded (Lado et al., 2018). In the current dataset, the highest performing Hybrid family from the SPACE study ($n = 32$) had a sward yield BLUP of +1.02 $\mathrm{Mg\,ha^{-1}}$. Applying the estimated correlation between flowering time BLUPs and sward yield BLUPs (a mean of 0.43 $\mathrm{Mg\,ha^{-1}\,day^{-1}}$; Table 3), then the Hybrid population in the training dataset ($n = 402$) contained dozens of genotypes with superior yield potential and four genotypes with twice the predicted sward yield GEBV of the highest ranked SPACE family. An additional facet to this strategy is the substantial genotype-by-environment effects previously reported in switchgrass (Boe & Casler, 2005; Casler, Sosa, et al., 2018; Casler, Vogel, et al., 2018; Cassida et al., 2005). By contrast, flowering time has a large but latitudinally predictable genotype-by-environment relationship with yield (Casler, 2019). Therefore, a breeding program could evaluate a large number of genotypes for flowering time at a single location and model their potential sward performance using yield trials at multiple sites.

5 | CONCLUSIONS

This study found that GP ability was not improved through sharing information between switchgrass breeding populations. Within breeding populations, flowering time GP can result in per-cycle breeding progress slightly less than previously reported phenotypic selection progress for flowering time (3.4 vs. 5 $\mathrm{day^{-1}\,cycle^{-1}}$) and sward biomass yield (0.44–0.67 vs. 0.71 $\mathrm{Mg\,ha^{-1}\,cycle^{-1}}$). Since cycles of GP cycles are shorter than phenotypic evaluation-based selection cycles, this indicates that GP can be a valuable tool to accelerate yield improvements in switchgrass breeding. Further, predictions based on Hybrid breeding population flowering time measurements could accurately predict sward yield performance in a parallel study (0.54–0.63 predictive ability), indicating potentially tight linkage between flowering time and biomass yield. Therefore, a MT breeding program sharing information between flowering time measurements and sward yield measurements could potentially result in rapid yield improvements in switchgrass.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data associated with this publication will be posted on Dryad Digital Repository (https://doi.org/10.5061/dryad.2jm63xss7). Field data is available for the primary training data and progeny dataset in CSV format. Marker data for individuals in this study is also provided in VCF format.

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REFERENCES

Alexander, L. W., Haynes, E. R., Burris, J., Jackson, S., & Stewart, C. N., Jr. (2014). Cultural treatments for accelerated growth and flowering of Panicum virgatum. *Biofuels*, *5*, 771–780. https://doi.org/10.1080/17597269.2015.1025466

Aravindhakshan, S. C., Epplin, F. M., & Taliaferro, C. M. (2010). Economics of switchgrass and miscanthus relative to coal as feedstock for generating electricity. *Biomass and Bioenergy*, *34*, 1375–1383. https://doi.org/10.1016/j.biombioe.2010.04.017

Arruda, M. P., Lipka, A. E., Brown, P. J., Krill, A. M., Thurber, C., Brown-Guedira, G., Dong, Y., Foresman, B. J., & Kolb, F. L. (2016). Comparing genomic selection and marker-assisted selection for Fusarium head blight resistance in wheat (*Triticum aestivum* L.). *Molecular Breeding*, *36*, 84. https://doi.org/10.1007/s11032-016-0508-5

Bhandari, H. S., Saha, M. C., Fasoula, V. A., & Bouton, J. H. (2011). Estimation of genetic parameters for biomass yield in lowland switchgrass (*Panicum virgatum* L.). *Crop Science*, *51*, 1525–1533. https://doi.org/10.2135/cropsci2010.10.0588

Bhatta, M., Gutierrez, L., Cammarota, L., Cardozo, F., Germán, S., Gómez-Guerrero, B., Pardo, M. F., Lanaro, V., Sayas, M., & Castro, A. J. (2020). Multi-trait genomic prediction model increased the predictive ability for agronomic and malting quality traits in barley (*Hordeum vulgare* L.). *G3: Genes, Genomes, Genetics*, *10*, 1113–1124. https://doi.org/10.1534/g3.119.400968

Boe, A., & Casler, M. D. (2005). Hierarchical analysis of switchgrass morphology. *Crop Science*, *45*, 2465–2472. https://doi.org/10.2135/cropsci2004.0703

Brandes, E., Plastina, A., & Heaton, E. A. (2018). Where can switchgrass production be more profitable than corn and soybean? An integrated subfield assessment in Iowa, USA. *GCB Bioenergy*, *10*, 473–488. https://doi.org/10.1111/gcbb.12516

Browning, B. L., Zhou, Y., & Browning, S. R. (2018). A One-penny imputed genome from next-generation reference panels. *American Journal of Human Genetics*, *103*, 338–348. https://doi.org/10.1016/j.ajhg.2018.07.015

Casler, M. D. (2019). Selection for flowering time as a mechanism to increase biomass yield of upland switchgrass. *Bioenergy Research*, *13*, 100–108. https://doi.org/10.1007/s12155-019-10044-3

Casler, M. D. (2020). Biomass yield evaluation for switchgrass breeding: Seeded swards vs. transplanted plots yield different results. *Bioenergy Research*. https://doi.org/10.1007/s12155-020-10214-8

Casler, M. D., & Brummer, E. C. (2008). Theoretical expected genetic gains for among-and-within-family selection methods in perennial forage crops. *Crop Science*, *48*, 890–902. https://doi.org/10.2135/cropsci2007.09.0499

Casler, M. D., & Ramstein, G. P. (2018). Breeding for biomass yield in switchgrass using surrogate measures of yield. *Bioenergy Research*, *11*, 1–12. https://doi.org/10.1007/s12155-017-9867-y

Casler, M. D., Sosa, S., Boe, A. R., & Bonos, S. A. (2018). Soil quality and region influence performance and ranking of switchgrass genotypes. *Crop Science*, *59*, 221–232. https://doi.org/10.2135/cropsci2018.06.0409

Casler, M. D., & Vogel, K. P. (2014). Selection for biomass yield in upland, lowland, and hybrid switchgrass. *Crop Science*, *54*, 626–636. https://doi.org/10.2135/cropsci2013.04.0239

Casler, M. D., Vogel, K. P., Lee, D. K., Mitchell, R. B., Adler, P. R., Sulc, R. M., Johnson, K. D., Kallenbach, R. L., Boe, A. R., Mathison, R. D., Cassida, K. A., Min, D. H., Crawford, J., & Moore, K. J. (2018). 30 Years of progress toward increased biomass yield of switchgrass and big bluestem. *Crop Science*, *58*, 1242–1254. https://doi.org/10.2135/cropsci2017.12.0729

Casler, M., Vogel, K., & Beal, A. (2006). *Registration of WS4U and WS8U switchgrass germplasms*. Publ. USDA-ARS UNL Fac. https://digitalcommons.unl.edu/usdaarsfacpub/1945

Cassida, K. A., Muir, J. P., Hussey, M. A., Read, J. C., Venuto, B. C., & Ocumpaugh, W. R. (2005). Biomass yield and stand characteristics of switchgrass in south Central U.S. environments. *Crop Science*, *45*, 673–681. https://doi.org/10.2135/cropsci2005.0673

Castro, J. C., Boe, A., & Lee, D. K. (2011). A simple system for promoting flowering of upland switchgrass in the greenhouse. *Crop Science*, *51*, 2607–2614. https://doi.org/10.2135/cropsci2011.03.0142

Covarrubias-Pazaran, G. (2016). Genome-assisted prediction of quantitative traits using the R package sommer. *PLoS One*, *11*, e0156744. https://doi.org/10.1371/journal.pone.0156744

Crossa, J., Pérez-Rodríguez, P., Cuevas, J., Montesinos-López, O., Jarquín, D., de Los Campos, G., Burgueño, J., González-Camacho, J. M., Pérez-Elizalde, S., Beyene, Y., Dreisigacker, S., Singh, R., Zhang, X., Gowda, M., Roorkiwal, M., Rutkoski, J., & Varshney, R. K. (2017). Genomic selection in plant breeding: Methods, models, and perspectives. *Trends in Plant Science*, *22*, 961–975. https://doi.org/10.1016/j.tplants.2017.08.011

Cuevas, H. E., Zhou, C., Tang, H., Khadke, P. P., Das, S., Lin, Y.-R., Ge, Z., Clemente, T., Upadhyaya, H. D., Hash, C. T., & Paterson, A. H. (2016). The evolution of photoperiod-insensitive flowering in sorghum, a genomic model for panicoid grasses. *Molecular Biology and Evolution*, *33*, 2417–2428. https://doi.org/10.1093/molbev/msw120

Cullis, B. R., Smith, A. B., & Coombes, N. E. (2006). On the design of early generation variety trials with correlated data. *Journal of Agricultural, Biological, and Environmental Statistics*, *11*, 381–393. https://doi.org/10.1198/108571106X154443

Cullis, B. R., Thomson, F. M., Fisher, J. A., Gilmour, A. R., and Thompson, R. (1996). *The analysis of the NSW wheat variety database. I. Modelling trial error variance*. https://pubag.nal.usda.gov/catalog/2467793

Dalid, C. O., Saxton, A. M., Allen, F. L., Pantalone, V., Nayak, S., & Bhandari, H. S. (2018). Genetic variation and expected per cycle biomass yield gain in lowland switchgrass. *Crop Science*, *58*, 1255–1264. https://doi.org/10.2135/cropsci2018.01.0026

Desta, Z. A., & Ortiz, R. (2014). Genomic selection: Genome-wide prediction in plant improvement. *Trends in Plant Science*, *19*, 592–601. https://doi.org/10.1016/j.tplants.2014.05.006

Endelman, J. B. (2011). Ridge regression and other kernels for genomic selection with R package rrBLUP. *Plant Genome*, *4*, 250–255. https://doi.org/10.3835/plantgenome2011.08.0024

Evans, J., Sanciangco, M. D., Lau, K. H., Crisovan, E., Barry, K., Daum, C., Hundley, H., Jenkins, J., Kennedy, M., Kunde-Ramamoorthy, G., Vaillancourt, B., Acharya, A., Schmutz, J., Saha, M., Kaeppler, S. M., Brummer, E. C., Casler, M. D., & Buell, C. R. (2018). Extensive genetic diversity is present within north American switchgrass germplasm. *Plant Genome*, *11*, 170055. https://doi.org/10.3835/plantgenome2017.06.0055

Fiedler, J. D., Lanzatella, C., Edmé, S. J., Palmer, N. A., Sarath, G., Mitchell, R., & Tobias, C. M. (2018). Genomic prediction accuracy for switchgrass traits related to bioenergy within differentiated populations. *BMC Plant Biology*, *18*, 142. https://doi.org/10.1186/s12870-018-1360-z

Fike, J. H., Pease, J. W., Owens, V. N., Farris, R. L., Hansen, J. L., Heaton, E. A., Hong, C. O., Mayton, H. S., Mitchell, R. B., & Viands, D. R. (2017). Switchgrass nitrogen response and estimated production costs on diverse sites. *GCB Bioenergy*, *9*, 1526–1542. https://doi.org/10.1111/gcbb.12444

Frichot, E., & François, O. (2015). LEA: An R package for landscape and ecological association studies. *Methods in Ecology and Evolution*, *6*, 925–929. https://doi.org/10.1111/2041-210X.12382

Gelfand, I., Hamilton, S. K., Kravchenko, A. N., Jackson, R. D., Thelen, K. D., & Robertson, G. P. (2020). Empirical evidence for the potential climate benefits of decarbonizing light vehicle transport in the U.S. with bioenergy from purpose-grown biomass with and without BECCS. *Environmental Science & Technology*, *54*, 2961–2974. https://doi.org/10.1021/acs.est.9b07019

Gianola, D. (2021). Opinionated views on genome-assisted inference and prediction during a pandemic. *Frontiers in Plant Science*, *12*. https://doi.org/10.3389/fpls.2021.717284

Glaubitz, J. C., Casstevens, T. M., Lu, F., Harriman, J., Elshire, R. J., Sun, Q., & Buckler, E. S. (2014). TASSEL-GBS: A high capacity genotyping by sequencing analysis pipeline. *PLoS One*, *9*, e90346. https://doi.org/10.1371/journal.pone.0090346

Heffner, E. L., Jannink, J.-L., & Sorrells, M. E. (2011). Genomic selection accuracy using multifamily prediction models in a wheat breeding program. *Plant Genome*, *4*, 65–75. https://doi.org/10.3835/plantgenome2010.12.0029

Jensen, E., Farrar, K., Thomas-Jones, S., Hastings, A., Donnison, I., & Clifton-Brown, J. (2011). Characterization of flowering time diversity in *Miscanthus* species. *GCB Bioenergy*, *3*, 387–400. https://doi.org/10.1111/j.1757-1707.2011.01097.x

Lado, B., Vázquez, D., Quincke, M., Silva, P., Aguilar, I., & Gutiérrez, L. (2018). Resource allocation optimization with multi-trait genomic prediction for bread wheat (*Triticum aestivum* L.) baking quality. *Theoretical and Applied Genetics*, *131*, 2719–2731. https://doi.org/10.1007/s00122-018-3186-3

Lenz, P. R. N., Nadeau, S., Mottet, M.-J., Perron, M., Isabel, N., Beaulieu, J., & Bousquet, J. (2020). Multi-trait genomic selection for weevil resistance, growth, and wood quality in Norway spruce. *Evolutionary Applications*, *13*, 76–94. https://doi.org/10.1111/eva.12823

Lipka, A. E., Lu, F., Cherney, J. H., Buckler, E. S., Casler, M. D., & Costich, D. E. (2014). Accelerating the switchgrass (*Panicum virgatum* L.) breeding cycle using genomic selection approaches. *PLoS One*, *9*, e112227. https://doi.org/10.1371/journal.pone.0112227

Lovell, J. T., MacQueen, A. H., Mamidi, S., Bonnette, J., Jenkins, J., Napier, J. D., Sreedasyam, A., Healey, A., Session, A., Shu, S., Barry, K., Bonos, S., Boston, L. B., Daum, C., Deshpande, S., Ewing, A., Grabowski, P. P., Haque, T., Harrison, M., … Schmutz, J. (2021). Genomic mechanisms of climate adaptation in polyploid bioenergy switchgrass. *Nature*, *1–7*, 438–444. https://doi.org/10.1038/s41586-020-03127-1

Masum, M. F. H., Dwivedi, P., & Anderson, W. F. (2020). Estimating unit production cost, carbon intensity, and carbon abatement cost of electricity generation from bioenergy feedstocks in Georgia, United States. *Renewable and Sustainable Energy Reviews*, *117*, 109514. https://doi.org/10.1016/j.rser.2019.109514

Moore, K. J., Moser, L. E., Vogel, K. P., Waller, S. S., Johnson, B. E., & Pedersen, J. F. (1991). Describing and quantifying growth stages of perennial forage grasses. *Agronomy Journal*, *83*, 1073–1077. https://doi.org/10.2134/agronj1991.00021962008300060027x

Nayak, S., Bhandari, H., Sams, C., Sykes, V., Hilafu, H., Dalid, C., Senseman, S., & Pantalone, V. (2020). Genetic variation for biomass yield and predicted genetic gain in lowland switchgrass "Kanlow". *Agronomy*, *10*, 1845. https://doi.org/10.3390/agronomy10121845

Perrin, R., Vogel, K., Schmer, M., & Mitchell, R. (2008). Farm-scale production cost of switchgrass for biomass. *Bioenergy Research*, *1*, 91–97. https://doi.org/10.1007/s12155-008-9005-y

Poudel, H. P., Sanciangco, M. D., Kaeppler, S. M., Buell, C. R., & Casler, M. D. (2019). Genomic prediction for winter survival of lowland switchgrass in the northern USA. *G3: Genes, Genomes, Genetics*, *9*, 1921–1931. https://doi.org/10.1534/g3.119.400094

Price, D. L., & Casler, M. D. (2014a). Divergent selection for secondary traits in upland tetraploid switchgrass and effects on sward biomass yield. *Bioenergy Research*, *7*, 329–337. https://doi.org/10.1007/s12155-013-9374-8

Price, D. L., & Casler, M. D. (2014b). Inheritance of secondary morphological traits for among-and-within-family selection in upland tetraploid switchgrass. *Crop Science*, *54*, 646–653. https://doi.org/10.2135/cropsci2013.04.0273

Price, D. L., & Casler, M. D. (2014c). Predictive Relationships between Plant Morphological Traits and Biomass Yield in Switchgrass. *Crop Science*, *54*, 637–645. https://doi.org/10.2135/cropsci2013.04.0272

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945–959. https://doi.org/10.1093/genetics/155.2.945

Ramstein, G. P., & Casler, M. D. (2019). Extensions of BLUP models for genomic prediction in heterogeneous populations: Application in a diverse switchgrass sample. *G3: Genes, Genomes, Genetics*, *9*, 789–805. https://doi.org/10.1534/g3.118.200969

Ramstein, G. P., Evans, J., Kaeppler, S. M., Mitchell, R. B., Vogel, K. P., Buell, C. R., & Casler, M. D. (2016). Accuracy of genomic prediction in switchgrass (*Panicum virgatum* L.) improved by accounting for linkage disequilibrium. *G3: Genes, Genomes, Genetics*, *6*, 1049–1062. https://doi.org/10.1534/g3.115.024950

Riedelsheimer, C., Endelman, J. B., Stange, M., Sorrells, M. E., Jannink, J.-L., & Melchinger, A. E. (2013). Genomic predictability of interconnected biparental maize populations. *Genetics*, *194*, 493–503. https://doi.org/10.1534/genetics.113.150227

Sanderson, M. A., Adler, P. R., Boateng, A. A., Casler, M. D., & Sarath, G. (2006). Switchgrass as a biofuels feedstock in the USA. *Canadian Journal of Plant Science*, *86*, 1315–1325. https://doi.org/10.4141/P06-136

Schmidt, P., Hartung, J., Bennewitz, J., & Piepho, H.-P. (2019). Heritability in plant breeding on a genotype-difference basis. *Genetics*, *212*, 991–1008. https://doi.org/10.1534/genetics.119.302134

Schmidt, P., Hartung, J., Rath, J., & Piepho, H.-P. (2019). Estimating broad-sense heritability with unbalanced data from agricultural cultivar trials. *Crop Science*, *59*, 525–536. https://doi.org/10.2135/cropsci2018.06.0376

Schulz-Streeck, T., Ogutu, J. O., & Piepho, H.-P. (2013). Comparisons of single-stage and two-stage approaches to genomic selection. *Theoretical and Applied Genetics*, *126*, 69–82. https://doi.org/10.1007/s00122-012-1960-1

Schwartz, C., & Amasino, R. (2013). Nitrogen recycling and flowering time in perennial bioenergy crops. *Frontiers in Plant Science*, *4*. https://doi.org/10.3389/fpls.2013.00076

Simeão Resende, R. M., Casler, M. D., & Vilela de Resende, M. D. (2014). Genomic selection in forage breeding: Accuracy and methods. *Crop Science*, *54*, 143–156. https://doi.org/10.2135/cropsci2013.05.0353

Taylor, M. S., Tornqvist, C.-E., Zhao, X., Grabowski, P., Doerge, R., Ma, J., Volenec, J., Evans, J., Ramstein, G. P., Sanciangco, M. D., Buell, C. R., Casler, M. D., & Jiang, Y. (2018). Genome-wide association study in pseudo-F2 populations of switchgrass identifies genetic loci affecting heading and anthesis dates. *Frontiers in Plant Science*, *9*. https://doi.org/10.3389/fpls.2018.01250

Tilhou, N. W., & Casler, M. D. (2021). Subsampling and DNA pooling can increase gains through genomic selection in switchgrass. *Plant Genome*, *14*, e20149. https://doi.org/10.1002/tpg2.20149

Tilhou, N. W., & Casler, M. (2022). Genetic correlations between switchgrass performance in sward conditions and surrogate measures. *Crop Science*, https://doi.org/10.1002/csc2.20767

Tilhou, N. W., Kaeppler, S. M., & Casler, M. D. (2022). Strong parallel evidence of selection during switchgrass sward establishment in hybrid and lowland ecotypes. *Grassland Research*, *1*(1), 31–42. https://doi.org/10.1002/glr2.12007

Uppalapati, S. R., Serba, D. D., Ishiga, Y., Szabo, L. J., Mittal, S., Bhandari, H. S., Bouton, J. H., Mysore, K. S., & Saha, M. C. (2013). Characterization of the rust fungus, *Puccinia emaculata*, and evaluation of genetic variability for rust resistance in switchgrass populations. *Bioenergy Research*, *6*, 458–468. https://doi.org/10.1007/s12155-012-9263-6

Van Esbroeck, G. A., Hussey, M. A., & Sanderson, M. A. (1998). Selection response and developmental basis for early and late panicle emergence in alamo switchgrass. *Crop Science*, *38*, 342–346. https://doi.org/10.2135/cropsci1998.0011183X003800020010x

VanWallendael, A., Bonnette, J., Juenger, T. E., Fritschi, F. B., Fay, P. A., Mitchell, R. B., Lloyd-Reilley, J., Rouquette, F. M., Jr., Bergstrom, G. C., & Lowry, D. B. (2020). Geographic variation in the genetic basis of resistance to leaf rust between locally adapted ecotypes of the biofuel crop switchgrass (*Panicum virgatum*). *The New Phytologist*, *227*, 1696–1708. https://doi.org/10.1111/nph.16555

Vogel, K., & Mitchell, R. (2008). *Heterosis in switchgrass: Biomass yield in swards*. Publ. USDA-ARS UNL Fac. https://digitalcommons.unl.edu/usdaarsfacpub/1960

Vogel, K. P., Mitchell, R. B., Casler, M. D., & Sarath, G. (2014). Registration of 'liberty' switchgrass. *Journal of Plant Registrations*, *8*, 242–247. https://doi.org/10.3198/jpr2013.12.0076crc

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution*, *38*, 1358–1370. https://doi.org/10.2307/2408641

Wright, L., & Turhollow, A. (2010). Switchgrass selection as a "model" bioenergy crop: A history of the process. *Biomass and Bioenergy*, *34*, 851–868. https://doi.org/10.1016/j.biombioe.2010.01.030

Zalapa, J. E., Price, D. L., Kaeppler, S. M., Tobias, C. M., Okada, M., & Casler, M. D. (2011). Hierarchical classification of switchgrass genotypes using SSR and chloroplast sequences: Ecotypes, ploidies, gene pools, and cultivars. *Theoretical and Applied Genetics*, *122*, 805–817. https://doi.org/10.1007/s00122-010-1488-1

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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