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Genetic Resistance of Switchgrass to Rust Evaluated in a Composite Upland × Lowland Population in Lab and Field Settings

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Abstract: Maintaining low levels of rust incidence (caused by *Puccinia novopanicci*) in switchgrass (*Panicum virgatum* L.) breeding populations is a priority for the USDA-ARS program engaged in improving cultivars for high biomass yield and quality. Essential to this goal is the unbiased and accurate estimation of genetic parameters to predict the merits of parents and progeny. Spores of the fungus were inoculated in greenhouse-grown seedling progeny of 31 half-sib families in generation 2 (Gen 2) of a composite Summer × Kanlow population for evaluation of rust incidence on the leaves with a 0–9 rating scale. Two parents were later chosen to cross and develop a linkage mapping population as Gen 3. The Gen 2, 3, and Kanlow seedlings were transplanted into the field located near Mead, NE, in early June 2020 and laid out as a replicated row–column design with six blocks of single-row plots of five plants each. The field trial was rated in September 2021 and 2022 with a 0–4 scale. Lab and field data were subjected to univariate linear mixed models via the restricted maximum likelihood to extract the variance components needed to predict the breeding values. The additive genetic variation was substantial ($p < 0.01$), enough to result in high heritability estimates ranging from 0.42 ± 14 to 0.73 ± 0.09 at the individual and family mean levels. This result implies that rust resistance is under strong genetic control to use mass selection for obtaining satisfactory gains. A possible rust incidence × year interaction was detected with a Spearman correlation of breeding values of -0.38 , caused by significant rank changes of the Gen 3 genotypes in 2022 (a high heat and drought year). Genetic gains were predicted to reduce rust incidence scores by at least two points on the rating scale when selecting backwards, and by one point when selecting individual candidates as parents of the next generation. Faster gains (31 and 59%) were realized relative to the second generation by respectively selecting the top 10% of the families in Gen 3 or the top 10% of genotypes within this group. Based on these results, strategies for controlling the incidence of rust will be developed to optimize gains in the other traits of economic importance.

Keywords: breeding values; ecotypes; half-sib; generation; genetic gains; genetic parameters; rust; switchgrass



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1. Introduction

Switchgrass (*Panicum virgatum* L.) is a multipurpose crop that drew greater interest from plant breeders, agricultural scientists, and lawmakers in the early 1990s to curb the use of fossil fuels in the world economy [1–3]. The wide adaptation to marginal environments provides switchgrass the capability to yield relatively high biomass with limited inputs. Combined with its perennial characteristics, the polyploid nature of switchgrass provides ample opportunities to breed this crop to meet the demands of clean fuels for a bioenergy industry [4,5]. The wide adaptation of switchgrass, however, relies on exploiting its existing ecotypic variation and geographic distribution to fit the crop to the many growing environments, ranging from tropical to temperate climates, with additional biotic and

abiotic stresses. The two main ecotypes (lowland and upland) show differential responses when exposed to these specific stresses, with antagonistic correlations between growth (biomass) and latitudes or between growth and biotic stresses [6]. The lowland ecotypes have a higher biomass yield and pest resistance at lower latitudes than the upland ecotypes, which mature earlier and offer a potential reservoir of genes for adaptation to below freezing temperatures [7].

One of the great successes of the USDA-ARS grass breeding program, based in Lincoln, NE (USA), was the 2014 release of Liberty [8], a cultivar that combines the high biomass yield of its paternal parent (the lowland Kanlow) with the overwintering capability of the maternal parent (the upland Summer). Switchgrass, being a polyploid, is very heterozygous, giving ample opportunity to select, within the Liberty (F1 hybrid) line, genotypes with different combinations of traits for specific adaptation [4]. Other breeding programs are exploiting this germplasm to select for later maturity and winter survival, aiming to increase biomass yield at higher latitudes [9]. The very high heterozygosity still requires greater emphasis to be put on the choice of candidate parents when it comes to controlling the incidence of genes for susceptibility to diseases or pests in this population. Recessive genes are masked in the heterozygous states and may be exposed in assortative matings with very closely-related parents.

Rust (caused by *Puccinia novopanicci*) is the most predominant disease of switchgrass with the potential to cause serious challenges to high biomass yield in the Central U.S. [10–13]. This breeding program considers integrating rust as a target trait in improving the Liberty population through advanced generations [4,7]. Cultivated switchgrass will be planted in large fields and will be exposed to more varying environmental conditions (temperatures and pests/pathogens) when plantations are expanded to supply biofuels. This makes the case for genetic resistance breeding as the most sustainable and cost-effective strategy to maintain the crop as a viable bioenergy and feedstock candidate. Several studies, using natural or artificial rust infections, have characterized the existing variation in switchgrass germplasm. All indicated that geographic variation forms the basis for rust resistance in switchgrass [14], existing both at the ecotype and at the within-population levels [15]. In a study based on leaf inoculation in petri dishes, [13] reported higher levels of resistance in ornamental than in cultivars bred for forage. Ma (2015) [10] reported differential responses of lowland and upland ecotypes to rust, with the former harboring the resistance genes. Gustafson et al. (2003) [16] indicated that both additive and nonadditive genetic variation were implicated in governing rust resistance in switchgrass. Epistatic genetic variation, coming from the interaction of two major loci, seems to be involved in imparting or explaining the association of quantitative trait loci (QTL) governing resistance [14]. More studies are needed at both the genetic and genomic levels to decisively decipher the inheritance of rust resistance and breed efficiently for the trait in switchgrass.

The main objective of this study was to provide a more comprehensive elucidation of the genetic control of rust resistance/susceptibility in switchgrass, scored in lab (seedling stage, artificial infection) and field (adult stage, natural infection) experiments, and to appraise the predicted and realized responses to selection. Genetic parameters were estimated aiming at understanding the mechanisms of inheritance of rust resistance in three generations of a composite switchgrass population.

2. Materials and Methods

2.1. Plant Materials and Experimental Design

Two experiments were conducted to evaluate rust resistance in the Summer $\times$ Kanlow (SK) switchgrass population. The first experiment tested, using artificial infection in a greenhouse, 31 half-sib families obtained by open pollination of parents from the second cycle of selection within the SK breeding population. Seeds of these families and of Kanlow and Summer were germinated on potting mix and maintained in a heated greenhouse. Five to six seedlings per family were raised individually in polyethylene cones, which were arranged in racks containing 98 (7×14) holes.

Urediniospores of *P. novopanici* were collected in gelatin capsules from previously infected switchgrass plants, dehydrated for 5–7 days in a desiccator, and finally stored at -80°C for eventual use as sources of inoculum ([17], submitted). Before use, the rust spores were transferred to microcentrifuge tubes and heat-shocked at 45°C for 10–15 min on a heating block. A rehydration step of the spores followed for about 3 h. Approximately 150 mg of hydrated urediniospores was suspended in 50 mL of sterile water that was mixed with a drop of Tween 20. Inoculations were carried out by spraying the seedlings (4th leaf stage) with an even coat of inoculum. Control plants were sprayed with distilled water premixed with Tween 20 only. The seedlings were then moved to a growth chamber (Convion A2000) and incubated for 48 h under 100% humidity (maintained with frequent mistings) and a 12 h light/dark cycle. After incubation, the seedlings, still in racks, were placed in tubs containing water, maintained in a greenhouse under an average day temperature of $25\text{--}26^{\circ}\text{C}$, and fertilized with a general-purpose fertilizer (20-20-20 NPK at 250 ppm) once a week.

Plants were scored for percent rust infection on the leaves at 10 days after inoculation, using a 0–9 rating scale [16] with 0 = no pustules to 9 = 50+% pustules. The ratings were used in statistical analyses to represent the greenhouse environment or experiment 1.

The second experiment included the same greenhouse seedlings (31 families), which were properly identified and kept in the greenhouse for another year. In 2019, a cross was made in the greenhouse between two plants of the Gen 2 generation to develop a full-sib population (Gen 3) for mapping quantitative trait loci in the regions associated with rust and biomass yield and quality. Seedlings from this cross and from experiment 1 were raised in the greenhouse until June of 2020, when they were transplanted into the field, located on the University of Lincoln of Nebraska ENREEC experiment station (lat. $41.145583^{\circ}\text{N}$, long. $-96.498611^{\circ}\text{W}$) near Mead, NE. The seedlings were planted in a row–column design, with 6 blocks of 5 plants per family plot and 25 rows across. Most families were replicated 2–4 $\times$ and nine out of the 31 were not. The mapping population (one full-sib family) was planted in 8 separate rows, interspersed among the 15 rows of seedlings from experiment 1 or Gen 2. The two remaining rows (8 and 16) were planted with seedlings of Kanlow. Plants were spaced 1.1 m apart within and between rows with no alleys between plots or blocks. Prior to transplanting, the field was sprayed with a cocktail of pre- and post-emergence herbicides (2.35 l ha^{-1} of Atrazine and Magnum and 1.2 l ha^{-1} of 2–4 D). Manual weeding was provided later to prevent weed competition that would suppress growth before winter. In successive years, the experiment was treated with the same herbicide cocktail and fertilized with 112 kg N ha^{-1} as Urea in early May. The individual genotypes were scored for natural infection of rust in early September of 2021 and 2022, by the same two raters, using a consensus score based on a scale going from 0 = no pustules to 4 = 50+% pustules.

2.2. Statistical Analysis

The two field ratings were compared with those from the greenhouse experiment as three separate traits. The individual data were analyzed separately with SAS v9.4 [18] as an incomplete block design and according to a linear mixed model:

$$\mathbf{y} = \mu + \mathbf{Xr} + \mathbf{Zb} + \mathbf{Zf} + \mathbf{Zfr} + \mathbf{e},$$

where $\mathbf{y}$ is the vector of phenotypic observations, μ is the overall mean, $\mathbf{r}$ is the vector of fixed replicate effects, $\mathbf{b}$ is the vector of random incomplete block effects within replication with an expectation of $\mathbf{b} \sim \text{N}(\mathbf{0}, \sigma^2_{\text{b}})$, $\mathbf{f}$ is the vector of random additive breeding values of all genotypes with $\mathbf{f} \sim \text{N}(\mathbf{0}, \sigma^2_{\text{f}})$, $\mathbf{fr}$ is the random interaction between replications and family effects (interpreted as plot effects) with $\mathbf{fr} \sim \text{N}(\mathbf{0}, \sigma^2_{\text{fr}})$, $\mathbf{e}$ is the random vector of residuals with $\mathbf{e} \sim \text{N}(\mathbf{0}, \sigma^2_{\text{e}})$; σ^2_{b} , σ^2_{f} , σ^2_{fr} , and σ^2_{e} are the respective variances for the effects. The $\mathbf{Z}$ are the design matrices that relate the random effects to the observations in $\mathbf{y}$.

The variance components were obtained with the restricted maximum likelihood method (REML) and utilized to extract the breeding values of all individuals present in

the experiment at the times of evaluations. Heritability estimates were extracted at the individual (h^2_i) and family mean (h^2_f) levels using the following formulas:

$$h^2_f = \sigma^2_a / \sigma^2_{Pf} = \sigma^2_f / r + \sigma^2_e / rbn$$

$$h^2_i = \frac{\sigma^2_a}{\sigma^2_{Pi}} = \frac{4\sigma^2_f}{\sigma^2_f + \sigma^2_b + \sigma^2_{fr} + \sigma^2_e}$$

where σ^2_{Pf} and σ^2_{Pi} are the respective phenotypic variances of family means and individual genotypes in the experiment; r , b , and n are the numbers of replications, blocks, and plants per family per replication. The other elements are as described above. Breeding values (BV) of individual and parent genotypes were predicted as best linear unbiased predictions (BLUP). Standard errors of all estimates were approximated using the Delta method [19].

The Spearman correlation coefficients (ρ) were obtained by regressing the breeding values of a trait on those of the other and used as surrogates for genetic correlations to explain rank changes, if any. As there were no significant differences among families using rust scores from the greenhouse assays, the lab data were not subjected to any downstream analysis beyond computing the variance components.

2.3. Responses to Selection

The strategy of this USDA-ARS breeding program is to select for both the parents in the crossing blocks, mainly for deployment, and for individual candidates in the progeny tests for advancement. The latter are measured with less precision, due to not being replicated, than the families which are in the progeny tests. In this respect, responses to selection were predicted on a family mean basis (to select parents in crossing blocks) and on an individual basis (to select parents for the next generation). Response to selection of families was calculated according to the following formula:

$$R_{fam} = 2i \times h^2_f \times \sigma_{Pf},$$

where h^2_f and σ_{Pf} are the heritability and phenotypic standard variation at the family mean level, and i is the standardized intensity of selection ($i = 1.755$ for a 10% selection differential).

Response to selection of individual genotypes was based on the formula:

$$R_i = i \times r_{PA} \times \sigma_a = i \times h_i \times \sigma_a,$$

where r_{PA} is the correlation between phenotype (rust score) and breeding values and represents, in other words, the accuracy of selection. As the information was derived from individual performance, r_{PA} is equivalent to the standard deviation of h^2_i [19,20].

Realized gains from selection were appraised relative to the mean of Kanlow or between the two generations. The BV were first adjusted (BV_adj) with the population mean as:

$$BV_adj = BV + \bar{Y}, \text{ with } \bar{Y} \text{ being the population mean.}$$

Gains (realized) from selecting families (Δ_{fam}) or individuals (Δ_{ind}) were estimated as follows:

$$\Delta_{fam} = 100 * (BV_{fam_adj} - \bar{y}) / \bar{y}$$

$$\Delta_{ind} = 100 * (BV_{ind_adj} - \bar{y}) / \bar{y},$$

with $\bar{y}$ being the best linear unbiased estimate (BLUE mean) of Kanlow. For so doing, the BV_adj of the group of seedlings derived from the greenhouse and in the mapping population (regarded as 2 different generations) were compared with the BLUE mean of Kanlow and against each other.

3. Results

3.1. Genetic Parameters

The analyses detected significant genetic variation ($p < 0.01$) among families for the two rust ratings measured in 2021 and 2022 in the field (Tables 1 and 2). The plot-to-plot effect was as important as the family variance to explain the phenotypic variation observed in the field. Family and plot effects each explain 17% of the phenotypic variance in 2021, but 10 and 4% in 2022, respectively. The greenhouse experiment did not reveal variation among families ($p = 0.19$) (Table 3). However, the interaction between family and replication effect was important, contributing 11% to the phenotypic variance. There was no effect ($p > 0.05$) due to replication in either laboratory or field experiment.

Table 1. Genetic variation in rust incidence in a Summer $\times$ Kanlow switchgrass population evaluated in the second and third generations in the field, near Mead, NE, in 2021.

Parameters	Estimate	Standard Error	Z-Value	p-Value
Family	0.244	0.102	2.39	0.008
Block (Rep)	0.083	0.066	1.25	0.105
Family $\times$ Rep	0.238	0.084	2.82	0.002
Residual	0.868	0.057	15.22	<0.0001
h^2_f	0.73	0.093		
h^2_i	0.68	0.25		

Table 2. Genetic variation in rust in Summer $\times$ Kanlow switchgrass population evaluated in the second and third generations in the field, near Mead, NE, in 2022.

Parameters	Estimate	Standard Error	z-Ratio	p-Value
Family	0.0956	0.03614	2.65	0.0041
Block (Rep)	0.1252	0.053	2.36	0.0091
Family $\times$ Rep	0.03521	0.03749	0.94	0.1738
Residual	0.6603	0.04287	15.4	<0.0001
h^2_f	0.63	0.09		
h^2_i	0.42	0.14		

Table 3. Genetic variation in rust incidence in a Summer $\times$ Kanlow switchgrass population evaluated as half-sib families in the second generation in the greenhouse, 11 days after inoculation in 2019.

Parameters	Estimate	Standard Error	z-Ratio	p-Value
Family	0.0844	0.09714	0.87	0.1924
Block (Rep)	0	-	-	-
Family $\times$ Rep	0.2944	0.1482	1.99	0.0235
Residual	2.2638	0.1749	12.94	<0.0001

Heritability estimates were high for natural infection by rust in both years (Tables 1 and 2). These estimates were relatively higher in 2021 ($h^2_f = 0.73 \pm 0.093$; $h^2_i = 0.68 \pm 0.246$) than in 2022 ($h^2_f = 0.63 \pm 0.098$; $h^2_i = 0.42 \pm 0.144$) at both the family mean and individual levels, respectively. The heritability values were measured with relatively good precision.

The Spearman correlation (ρ) indicated significant rank changes in rust infection from one year to the next. Using the adjusted family breeding values, $\rho = 0$, suggesting that the 2021 data could not exactly predict the rust scores for the following year (2022). The mean BV rating ranged from 0.16 to 2.91 in 2021, and from -0.54 to 3.31 in 2022. Using the adjusted breeding values for individual genotypes, $\rho = -0.38$, indicating that genotypes

with high ratings in 2021 tended to have the lowest values in 2022, and vice versa. The BV of individual genotypes ranged from -0.34 to 3.63 in 2021 and from -1.65 to 4.19 in 2022.

3.2. Predicted and Realized Responses to Selection

Selecting backwards for parents on the tested families would reduce rust scores by three or two points, respectively, using ratings from either year (Table 4). Selection of individual candidates as parents of the next generation would decrease rust incidence in the field by 1.43 or 0.70 points using data taken in 2021 and 2022, respectively. Kanlow was very consistent in its reaction to field exposures to rust, with a mean BV of -2.52 (range: -3.16 to -0.18) in 2021 and of -1.75 (range: -2.2 to -1.2) in 2022. The BV of the Gen 2 families averaged 0.22 (range: -0.8 to 1.4) in 2021 and -0.013 (range: -0.4 to 0.7) in 2022, whereas the BV of their progeny ranged from -2.08 to 2.27 in 2021 and from -1.01 to 1.20 in 2022. The mean BV of the mapping population (a full-sib population representing Gen 3) was -0.89 in 2021 and 1.05 in 2022, displaying BV in its progeny ranging from -2.75 to 0.97 in 2021 and from 0.35 to 1.76 in 2022. As realized genetic gains, the two groups (Gen 2 and 3) were, respectively, 72% and 64% less effective at combating the rust infection than Kanlow in 2021. However, the reactions to rust of the top 10% within each of these groups were 51% and 81% as effective as that of Kanlow in 2021. In 2022, the reactions of Gen 2, as a group and individually, were consistent with the 2021 performance. The reactions of the mapping population were completely different in 2022, with a decrease in effectiveness of 151% as compared with that observed in 2021 (Figures 1 and 2). Using the 2021 data, selecting on Gen 3 would realize gains of 31% and 59% over Gen 2 based on family and individual levels.

Table 4. Realized and predicted gains in rust resistance measured in a Summer $\times$ Kanlow switchgrass population as evaluated in the 2nd and 3rd generations in the field, near Mead, NE, in 2021 and 2022.

Populations	BV ^a (Mean)	Gains_Pred ^b	Top 10% ^c	Gains_Pred
Δ_{fam} -2021		3.06		
Δ_{fam} -2022		2.12		
Δ_i -2021		1.43		
Δ_i -2022		0.70		
Kanlow	-2.452			
Gen 2 _{-family}	0.21^d	-1.08	-0.68	-0.72
Gen 2 _{-individual}			-1.25	-0.49
Gen 3 _{-family}	-0.889	-0.64	-0.889	-0.64
Gen 3 _{-individual}			-1.99	-0.19
Gains_realized (%)				
Gen 3/2 _{-family}	31			
Gen 3/2 _{-individual}	59			

^a Mean breeding values of the families. ^b Gains predicted as per the formulas: $R_{fam} = 2i \times h^2_f \times \sigma_{Pf}$; $R_i = i \times r_{PA} \times \sigma_a = i \times h_i \times \sigma_a$. ^c Calculated in the top 20% of the families or individual genotypes within a generation.

^d Calculated relative to Kanlow mean breeding values.

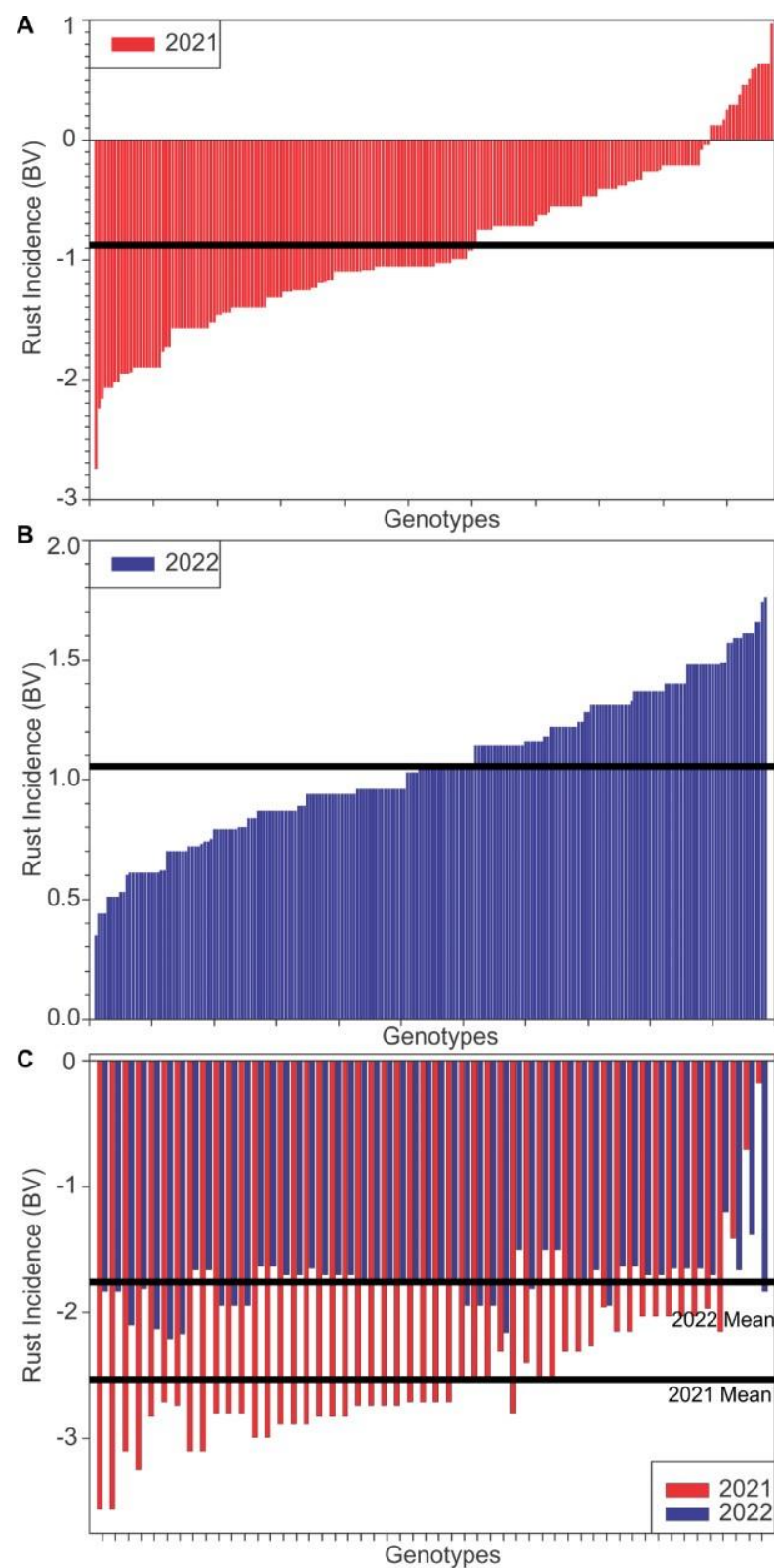


Figure 1. Distributions of breeding values for natural rust incidences predicted on two generations of a Summer $\times$ Kanlow switchgrass population. (A) Mapping population tested in 2021. (B) Mapping population tested in 2022. (C) Kanlow population tested in 2021 (blue bars) and 2022 (red bars). The horizontal bars represent the means of the populations. Families and progeny were rated on a 0–4 scale in a field, near Mead, NE.

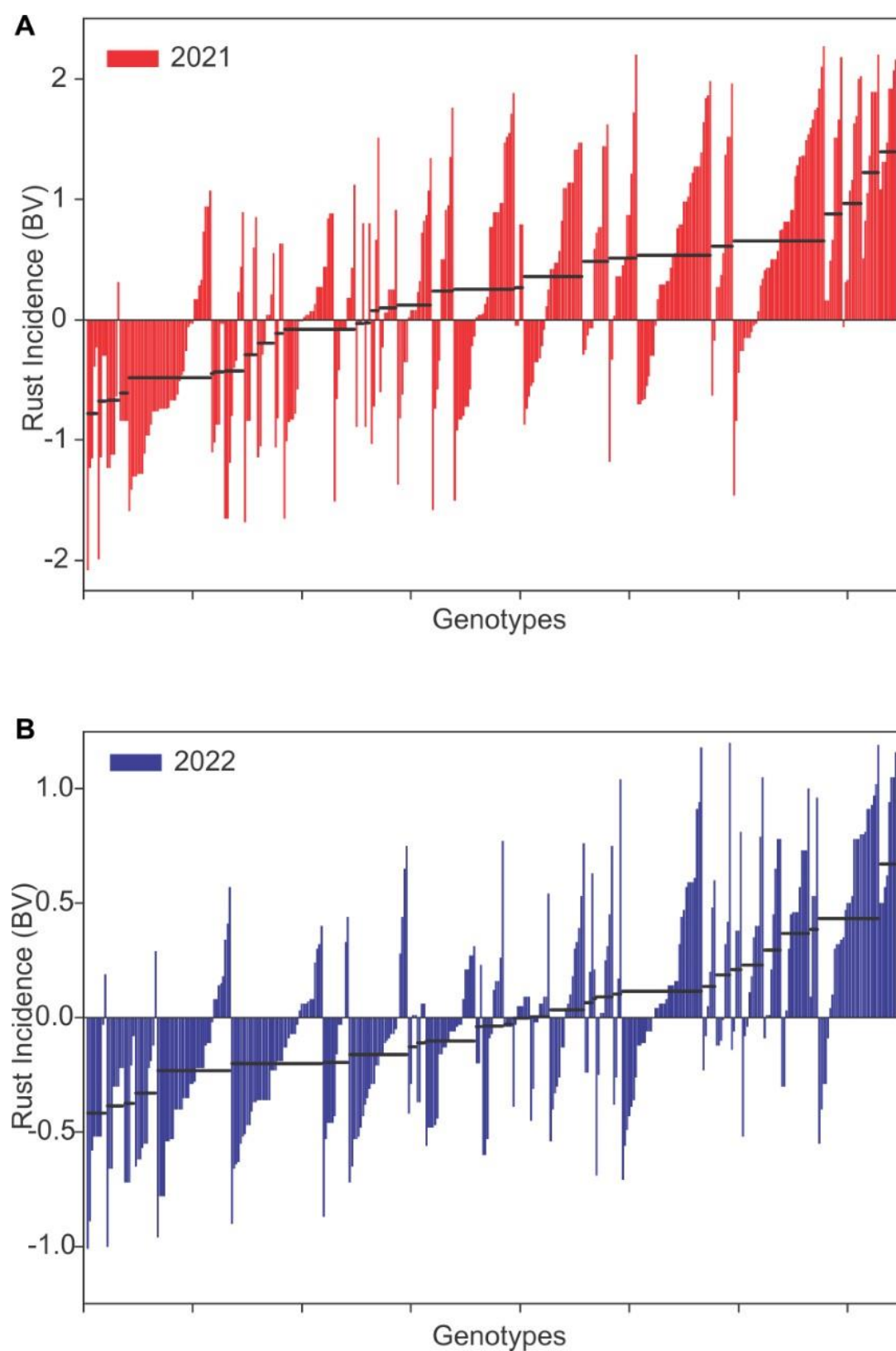


Figure 2. Distributions of breeding values for natural rust incidences predicted on generation 2 of a Summer × Kanlow switchgrass population. (A) Half-sib families with progeny tested in 2021. (B) Half-sib families with progeny tested in 2022. The black horizontal bars represent the means of the families with respective progeny as a group (width of the bars). Families and progeny were rated on a 0–4 scale in a field, near Mead, NE.

4. Discussion

The USDA-ARS perennial grass program in Lincoln, NE, applies a recurrent breeding and selection strategy to breed perennial grasses for the different traits deemed to be of economic importance. This breeding program is the only one engaged in a long-term commitment to simultaneously improve rust resistance with production traits in switchgrass and, as such, there are not many estimates available for comparison with the results obtained here. The strong emphasis on rust resistance is novel and aims at maintaining infections in switchgrass cultivars to levels close to those in Kanlow, i.e., with the majority of genotypes having no infections and a few having at least a rating score of 1. In a way, this infection level would not put strong pressure on the rust pathogen to change its genetic makeup too quickly. This study investigated the genetic control of rust infections in a composite switchgrass population developed by crossing a susceptible cultivar (Summer) as female and a resistant cultivar (Kanlow) as male. The cross was originally intended to improve yield and quality traits in the context of bioenergy, not rust resistance. Due to the female parent being the susceptible side of the equation, it is relevant to exert, by breeding, control on rust infections in this population throughout advanced generations.

The laboratory assays did not detect any significant differences among the families involved in the tests and these data were dropped from further analyses. The ratings used were taken 10 DAI, which may have been too early to have comparable performance with field ratings. A longer period of incubation and evaluation may need to be defined to exploit laboratory assays on switchgrass that can predict field reactions to rust for breeding purposes, particularly in the context of genomic selection. The assays were adequate to discriminate the proteomic responses to rust in Summer and Kanlow, but Kanlow took a considerably longer time to show any symptoms [17].

This program breeds for general combining ability, using open pollination to recombine genes/alleles in the parents for each generation [4]. This strategy, to be successful, relies on sampling ample additive genetic variation in the breeding population. The switchgrass population, as structured for this study and observed for two years, displays significant additive genetic variation for rust resistance with high heritability estimates (range: 0.42–0.73) at both the family mean and individual plant levels. This suggests that this breeding population offers ample opportunities to maintain rust infections at very low levels through breeding. The combined studies by [21,22] have reported heritability estimates as high as 0.96 for rust in some populations and as low as 0 in others, and considerable additive genetic variation. Comparing the family and individual estimates in the present study further suggests that dominance genetic variation may play a minimal role in switchgrass rust resistance, contrary to the findings of [16]. However, the present study was undertaken in only one environment, and these estimates may have been overestimated. A diallel experiment across locations may need to be designed to separate these two components of genetic variation.

The heritability estimates were slightly lower in 2022 than in 2021. The year 2022 was characterized by a severe drought and heat at the experiment station and throughout Nebraska. This made 2022 in NE less conducive than 2021 to rust infections, which require relatively high humidity for the pathogen to multiply and challenge the genotypes [23]. Harvell et al. (2002) [24] and [25] indicated that weather events are important drivers of disease incidences that will be aggravated by climate change. In Eucalyptus (*E. grandis*), rust incidence and heritability estimates were higher in humid environments, with heritability showing a linear increase with mean rust incidence, or lower under heat and drought conditions [26]. In Iowa, a state neighboring Nebraska to the East, rust incidence caused by *P. novopanici* was reported to vary from year to year, whereas in South Dakota, a neighboring state to the North, high incidence was reported for about the same period [27]. These fluctuations in rust incidence and weather will be common, and entail that rust incidence must be evaluated over more than a year for breeding purposes. However, stable cultivars and germplasm can be designed by selective breeding.

Kanlow and the genotypes in Gen 2 were very stable in their reactions to rust (Figures 1 and 2). Based on the distributions of the breeding values, the mapping population was somewhat more exacerbated in 2022 with higher levels of rust (Figure 1A,B). Kanlow and the families in Gen 2 as synthetic populations may be more buffered against the weather vagaries, as indicated in this drought by rust interaction (very low and negative ρ when using the individual datapoints). The mapping population, on the contrary, is a biparental population made by crossing two genotypes from Gen 2, a susceptible with a resistant genotype. As such, it does not encompass, by itself, the full array of genotypes in a generation, nor is it a typical half-sib family used in the progeny tests. It may be necessary to supplement the field site with overhead irrigation in the following years, just enough to maintain humidity at the leaf level, if drought, associated with high heat, is a concern.

Benchmarking on Kanlow as a reference cultivar for high biomass yield and low rust incidence is appropriate to improve the SK population for both traits. Summer, at least, needs to be included as the susceptible founder of this composite population and as a low biomass yielder in this NE environment. Some Summer seedlings will be added next year as rust references. There was still substantial variation within each of these populations included in the experiment for individual selection to meet the rust resistance/tolerance objective. The spreads of rust incidence in these individual populations show that Kanlow might have introgressed resistance genes in the composite population (Figures 1 and 2). Using a reciprocal Lowland $\times$ Upland F2 population for QTL mapping, Milano found a resistant allele from the lowland background that was responsible for rust resistance. While the rust scores extend in both years to the highest end of the spectrum (rating = 4), the breeding values were shrunk towards the mean, which is a typical and desirable property (the “shrinkage effect”) of the BLUP procedure [28,29]. The fact that the individual genotypes exhibited a larger variation than the families corroborates the between- then within-family selection strategy adopted in the breeding program [4]. The significant family $\times$ replication reported in this study and in that of [16] was to be expected and justifies the within-family selection. This plot-to-plot variation can be explained by the fact that, as a polyploid, each individual progeny in a switchgrass family, be it full-sib or half-sib, represents a distinct genotype with its specific reaction to rust, as determined by its sampling of the Mendelian genetic variance (random sampling of alleles from either parent). In their genetic study, [16] also concluded that selecting the most rust-resistant progeny within families is the best approach to control the incidence by breeding. The two years of biomass harvests have not yet been reached to allow an appraisal of the genetic correlations between rust incidence and biomass yield.

To make greater gains from selection and reduce the breeding cycle by 1–2 years, the USDA-ARS breeding program in Lincoln has adopted a strategy of using the progeny tests to evaluate, as the name implies, the parents in the crossing blocks for their genetic merits and deployment for cultivars. The progeny are selected in another year to be advanced as parents of the next generation. Based on the 2021 rust data, genetic gains were predicted to reduce rust incidence scores by at least two points on the rating scale when selecting backwards for deployment, and by one point when selecting individual candidates as parents of the next generation (Table 4). Faster gains (31% and 59%) were realized relative to the second generation by respectively selecting the top 10% of the families in Gen 3 or the top 10% within this group. This is an indication that Kanlow has introgressed positive alleles/genes for resistance into this hybrid population [30], and substantial gains are possible for this trait by increasing the frequency of these alleles with recurrent selection and selective breeding. Serba et al. (2015) [31], using RNA-seq, have pinpointed the nucleotide binding site–leucine-rich repeat domain, containing disease resistance genes, as playing an important role in resistance to rust in switchgrass. Moreover, the gene transcripts were identified and their inheritance traced in the three-generation pedigree studied by transcriptome analysis. The conflicting results in 2022 (rust $\times$ year interaction as Gen 3 $\times$ year) will be investigated for a longer period of time by applying overhead irrigation, when needed, in times of severe drought and heat. Integrating the pedigree into

the analysis may also help in understanding the uneven reaction of the Gen 3 family, based on its connectedness with other individuals in the previous generation.

5. Conclusions

This study investigated the estimation of genetic parameters for rust resistance, aiming at implementing a breeding and selection strategy to control the disease in future generations. The results indicated that rust incidence in switchgrass is genetically controlled as a highly heritable trait with ample opportunity to make gains from selection. Even though the study detected a possible rust incidence-by-year interaction, the reductions in heritability estimates were relatively small. This interaction may require a longer-term study and some overhead irrigation in times of drought and high heat to maintain a high inoculum level in the field. The results can be taken as evidence that Kanlow has introduced positive alleles for rust resistance in the SK hybrid population and that it is feasible to maintain low levels of infections with selective breeding. In this context, a reciprocal recurrent selection and breeding strategy was implemented to capitalize on the complementary gene actions of the two parental populations to simultaneously improve yield and quality traits with diseases and overwintering tolerance. A genomic selection program was also initiated to map the quantitative trait loci and optimize selection efficiency for these traits.

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