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Samikshya Rijal
University of Georgia
Shiva O. Makaju
University of Georgia
Mitra Mazarei
University of Tennessee at Knoxville
Jazib A. Ali
University of Georgia
C. Neal Stewart
University of Tennessee at Knoxville
Ali M. Missaoui
University of Georgia
James W. Buck
University of Georgia
Bochra A. Bahri
bbahri@uga.edu
University of Georgia

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Selection for multi-environment stability of rust, Bipolaris leaf spot, and false smut disease resistance in switchgrass (*Panicum virgatum* L.).

Samikshya Rijal[1], Shiva O. Makaju[2,3], Mitra Mazarei[4,5], Jazib A. Irfan[2], C. Neal Stewart Jr[4,5], Ali M. Missaoui[2,3], James W. Buck[6], and Bochra A. Bahri[1,6*]

[1]Institute of Plant Breeding, Genetics, and Genomics, University of Georgia, Griffin, GA 30223, USA

[2]Institute of Plant Breeding, Genetics and Genomics, University of Georgia, Athens, GA 30602, USA

[3]Department of Crop and Soil Sciences, University of Georgia, Athens, GA 30602, USA

[4]Department of Plant Sciences, University of Tennessee, Knoxville, TN 37996, USA

[5]Center for Agricultural Synthetic Biology, University of Tennessee, Knoxville, TN 37996, USA

[6]Department of Plant Pathology, University of Georgia, Griffin, GA 30223, USA

*Corresponding author: Bochra A. Bahri (bbahri@uga.edu)

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Abstract

Switchgrass (*Panicum virgatum L.*) is a warm-season, perennial, herbaceous feedstock crop for bioenergy that is threatened by diseases that reduce biomass yield. To address the need for resilient germplasm in breeding programs, a population of 200 half-sib families, derived from 50 accessions, was evaluated across three locations in Georgia and Tennessee over two years (2023 to 2024). Genetic variation for resistance to rust, Bipolaris leaf spot (BLS), and false smut (FS) was quantified, and high-performing families were identified. Mixed-model analysis was utilized to generate best linear unbiased predictions (BLUPs) and stability of genotype by environment interaction was assessed using the additive main effects and multiplicative interaction (AMMI) model. Ranking based on BLUP- and AMMI-based stability values helped to determine the genetic merit of families. High broad-sense heritability was observed for rust (0.77), BLS (0.59), and FS (0.78). Rust and BLS were strongly correlated (0.32 to 0.73) and showed a negative correlation with biomass yield (-0.12 to -0.53). BLUP, and AMMI analysis identified the top 20 families that were stable performers across environments; eleven families provided resistance to at least two of the diseases studied. These families can be important resources in switchgrass breeding.

Keywords: Disease resistance, Rust, Bipolaris leaf spot, false smut, phenotypic selection, stability

Introduction

Switchgrass was selected by the U.S. Department of Energy as a major feedstock for biofuel, especially for developing sustainable aviation fuel (SAF). It is a good alternative to fossil fuels due to its effectiveness in generating renewable energy while helping with carbon sequestration [1]. The perennial growth habit and high biomass yield potential even in low-input conditions make switchgrass an ideal candidate for sustainable feedstock production [2]. Additionally, as a native species to North America, the availability of a broad genetic diversity provides germplasm resources to develop switchgrass varieties suitable for various growing areas across the USA [3, 4]. Switchgrass is broadly categorized into three ecotypes that differ in growth habit, adaptation, and ploidy level: upland, lowland, and coastal [3, 5].

Although resilient in nature, the monocropping system and dense planting of switchgrass may contribute to infection by several fungal diseases, including rust, head smut, false smut (FS), and Bipolaris leaf spot (BLS) [6–10]. Rust is the most common disease, with most germplasm being susceptible [7, 8]. Head smut has been reported mostly in northern states and Texas [6, 9, 11]. BLS has been reported to be an emerging threat in New York and Tennessee [12, 13], and false smut in Georgia [10]. Disease resistance is important to maintain yield in perennial stands. The goal of biofuel production for transportation, including SAF, can be met only with the establishment of sustainable production systems that include disease management. Management of diseases through cultural practices such as crop rotation is problematic due to the perennial growth habit of switchgrass. Host resistance therefore becomes a central management tool.

Disease management through durable resistance requires knowledge of pathogen biology, disease prevalence, population structure, aggressiveness, and temporal shifts in pathogen races or species [14]. Resistance genes or marker trait associations can be identified with genome wide

association studies (GWAS) and other quantitative trait locus (QTL) mapping methods and be used to develop marker-assisted selection (MAS) strategies. Two large-effect and many small-effect QTLs were associated with rust resistance within a switchgrass diversity panel evaluated across northern and southern field locations in the USA [9, 15]. Two of these QTLs (*Prr1* and *Prr2*) had major effects in northern environments, whereas rust resistance in southern locations appeared to be polygenic. MAS contributing 3-26% phenotypic variance was reported for BLS resistance in switchgrass in a northern association panel containing upland ecotypes [12, 16]. No resistance loci have yet been reported for head smut and false smut. Despite these efforts, disease resistance markers have not been developed for the selection of resistant genotypes.

While availability of markers helps accelerate selection, phenotypic-based selection continues to provide a fundamental basis for identifying and advancing superior genotypes in breeding programs. Phenotypic recurrent selection is performed by evaluating a population, selecting the best individuals, intercrossing them to form the next generation, and repeating the process to accumulate favorable alleles at each breeding cycle for developing cultivars in switchgrass [17]. A well-documented recurrent section in switchgrass is the two step between and within family selection (BWFS) strategy [18, 19]. These steps include evaluating switchgrass populations at the family level, selecting among families, and then selecting the best individuals within families. The BWFS approach has not been utilized for disease resistance in switchgrass.

An accurate estimation of genetic merit for phenotypic recurrent selection is essential when populations are evaluated across multiple environments, where performance is influenced not only by genetics but also by environmental effects and genotype x environment (G × E) interactions [20]. To account for these factors, families can be ranked using either raw means or

best linear unbiased predictors (BLUPs) derived from mixed linear models. BLUPs provide improved estimates of genetic effects by accounting for unbalanced data, replication structure, and environmental heterogeneity, thereby enhancing selection accuracy in large, multi-environment field trials [21]. Since G × E interactions can cause family rankings to shift across locations and years, further analysis of environmental response patterns is necessary [22].

Partitioning of G × E into main effects (genotype and environment means) and interaction principal components (IPCAs) can be done by additive main effects and multiplicative interaction (AMMI) analysis [23]. Environmental stability analysis identifies broadly adapted versus environment-specific families for the traits. Stability of genotypes/families across environments can be estimated using AMMI stability values (ASVs), which represent the distance from the AMMI biplot origin. Lower ASV values correspond to more stable genotypes. Furthermore, BLUP and ASV can be used to identify the best families for the next breeding cycle [24]. Selection indices developed by integrating ASV with BLUP values, has been used to select for drought adaptation and biomass yield in switchgrass [19, 25], to improve grain yield in crops like wheat, barley and finger millet [26–28] and disease resistance in wheat (rust), maize (late wilt), and mungbean (*Boeremia* leaf spot) [26, 29, 30]. ASV integrated selection has yet to be employed in disease related traits in switchgrass.

The University of Georgia switchgrass breeding program aims to develop low-input, high biomass-yielding cultivars with drought tolerance and disease resistance. To achieve this goal, a diversity panel of switchgrass accessions was evaluated for biomass yield, nitrogen use efficiency, cell wall composition, and drought adaptation [25, 31–33]. A half-sib population was then generated for family-based selection [19]. Since the establishment of this population, multiple diseases have been recurrently identified at field locations, including rust, BLS and

ergot caused by *Puccinia novopanici, Bipolaris oryzae, Claviceps clavispora*, respectively. We performed phenotypic selection for disease resistance using two years of evaluation of this half-sib population across three locations, following the approach previously applied for biomass yield evaluation by Irfan et al., [19]. The objective was to identify top families that are resistant to the prevalent diseases in switchgrass and to perform stability analysis to select the best families for breeding for disease resistance.

Materials and Methods

Population and experimental design

The switchgrass half-sib population used for this study was previously evaluated for biomass and stability analysis by Irfan et al., [19]. This population consists of 200 half-sib families derived from a selected set of accessions from a switchgrass diversity panel [25, 32, 33]. From the diversity panel, 50 high-yielding accessions representing early- and late-flowering phenotypes were clonally propagated and planted in two crossing blocks separated by sorghum as a barrier, with each block arranged in a randomized complete block design with four replications [19]. The individuals from 200 half-sib families (f1-200 x 4 seeds from each = 800 individuals designated as FID1-800) were clonally propagated and established at three field locations (Fig. S1 in Online Resource 1). The clones were transplanted in 2021 at two field sites in Georgia: the University of Georgia Iron Horse Plant Sciences Farm (IHF) in Watkinsville (33°43′15.7″ N, 83°18′37.7″ W) and Gibbs Farm (TIF) in Tifton (31°43′8.43″ N, 83°58′0.08″ W), and in 2022 in Tennessee, at the University of Tennessee's East Tennessee Research and Education Center (TN) in Knoxville

(35°54'11.4" N; 83°57'33.31" W). The 200 half-sib families (with four plants per family) were randomized across two replications per location. Plots were spaced 1.52 m between rows, 1.52 m between families, and 1 m between plants within families.

Disease surveillance and phenotyping

Disease severities were recorded for rust in all three locations (IHF, TIF, TN), BLS in IHF and TIF, and FS in TN, in 2023 and 2024 (Table S1 in Online Resource 2). Diseases occurred from natural infections and were scored using a 0–9 scale, with 0 = no infection and 9 = 90-100% of foliage infected [7, 34]. Rust severity data were collected over two years at TN (rust_TN23 and rust_TN24), two years at IHF (rust_IHF23 and rust_IHF24), and one year at TIF (rust_TIF23). Likewise, BLS severity was collected for two years at two sites in Georgia (BLS_TIF23, BLS_TIF24, BLS_IHF23, and BLS_IHF24), and false must severity was scored only at TN for two years (FS_TN23, FS_TN24). The switchgrass fields were surveyed monthly from April to November, and severity scoring was conducted at one time point (late July–early August at TIF, late August at IHF, and late September at the TN site).

Variance component analysis and broad-sense heritability (H^2)

The distributional properties of phenotypic data were evaluated. Normality of observations at the individual, family, and parental (maternal)-levels was assessed using the Shapiro–Wilk test (Table S2 in Online Resource 2) and visualized with quantile–quantile (Q–Q) plots. For the three disease traits, linear mixed models (LMMs) were fitted at the parental, family, and individual

levels using the lme4 package in R [35]. Model assumptions were evaluated through residual diagnostics, including assessment of residual normality using Q–Q plots and the Shapiro–Wilk test, and homogeneity of variance using residuals versus fitted value plots.

The linear mixed model was fitted as [19],

$$Yijkl = \mu + Ei + Rj(i) + Fk + (FE)ik + \varepsilon ijkl \quad (1)$$

Where μ is the grand mean, *Ei* is fixed effect of *i*-th environment, *Rj(i)* is random effect of replication nested within environment, *Fk* is random effect of the *k*-th family, *(FE)ik* is random interaction effect between the *k*-th family and *i*-th environment, and *εijkl* is the residual error. For parent and individual models, the family terms from the model (*F*) were replaced with the respective, parental (*P*) and individual (*FID*) terms.

Variance components from mixed models were used to estimate broad-sense heritability (H^2). The H^2 estimate at the individual level was defined as clonal repeatability, representing the proportion of phenotypic variation due to the total genetic identity of a single plant (including both family and individual effects). The parental-level H^2 was calculated to assess the genetic merit of founding lines, while family-level heritability was used to estimate the reliability of family mean performance across environments.

For each disease, H^2 was estimated as [36],

$$H^2 = \sigma^2 g/(\sigma^2 g + \sigma^2 ge/n + \sigma^2 \varepsilon/nr),$$

where $\sigma^2 g$ is the variance component for the genotype (parent, family, individual), $\sigma^2 ge$ is the variance component for genotype-by-environment interaction, and $\sigma^2 \varepsilon$ is the residual variance, *n* is the number of environments, *r* is the number of replicates per environment.

176

177 **Correlations between disease and yield traits**

178 To understand the relationship between disease traits and biomass yield, correlation analyses
179 were performed using *Hmisc* package in R [35]. Pearson's correlation coefficients were
180 calculated to estimate correlations based on family BLUPs for rust, BLS, and FS, and biomass
181 yield. Biomass yield data from Irfan et al., [19] collected in 2023 and 2024 for the same
182 population as this study was used. To account for the hierarchical structure of the half-sib
183 families, correlations were calculated at the family-level (200 families) and parental-level (50
184 parental accessions) between environments.

185

186 **2Multi-environment stability analysis**

187 *BLUP-based selection of candidates for stability analysis*

188 The 200 half-sib families were ranked based on their predicted disease severity values, calculated
189 as the sum of the environmental and family BLUPs derived from a family-based mixed model
190 (Equation 1). Simultaneously, a parental model was fitted in which the family term (*Fk*) in
191 Equation 1 was replaced by a parental term (*Pk*), allowing ranking of the 50 maternal parents.
192 Selection of superior families was performed by identifying 20 half-sib families using three
193 criteria (Table S3 in Online Resource 2): (i) lower disease severity BLUP, indicating high disease
194 resistance, (ii) maximum representation of distinct parents among the selected families, and (iii)
195 inclusion of families with high biomass yield potential based on rankings reported by Irfan et al.,
196 [19]. To analyze the relative performance of the selected top-performing families, BLUP

deviations were calculated by subtracting the population's grand mean from the family-specific BLUPs.

AMMI analysis of variance

Environment-specific BLUPs from the top 20 half-sib families for rust and BLS disease were organized into a G × E matrix (Table S4 in Online Resource 2) and used for multi-environment stability analysis in GenStat 24 [37]. Stability analysis for FS was not performed as data was only available from two environments at the TN site. The AMMI model was applied to partition total phenotypic variation into genotype (family), environment, and G × E interaction components, followed by principal component analysis of the interaction matrix. The AMMI analysis of variance tested the significance of genotype, environment, and G × E interaction effects. Interaction effects were further decomposed into interaction principal component axes (IPCA1 and IPCA2). Stability of family performance across environments was quantified using the AMMI stability value (ASV), calculated from IPCA1 and IPCA2 scores, where lower ASV values for disease severity indicate greater stability. Interaction patterns among families and environments were visualized using AMMI biplots (IPCA1 vs IPCA2). Visualization based on BLUP values resulted in substantial overlap among families; therefore, final visualization was performed using raw severity data, with AMMI analysis conducted using the *metan* package in R [38].

Combined ranking (CR) based on BLUP and ASV

To evaluate disease severity and stability, ranking for both rust and BLS were calculated by considering disease severity BLUPs for each environment with their ranks, and the ASV rank, by summing all those ranks. The CR of each genotype represents the sum of ranks of severity BLUP from each environment and the rank of ASV. Lower CR values indicate superior and more stable disease resistance. This stability ranking is based on previously published yield-weighted stability index (YWSI) by Irfan et al., [19] and is different from the AMMI stability index (ASI), as it uses overall BLUP from all environments pooled and ASV. As the performance of each environment is considered, compared to one-time overall performance across the environments in ASI along with ASV, these results provide better support for selection decisions.

Results

Disease surveillance and distribution

Rust, BLS, and FS disease were observed across all field sites during both years. Disease severity was recorded on a 0-9 ordinal scale across multiple environments: five for rust (TIF24, IHF23, IHF24, TN23, and TN24), four for BLS (TIF23, TIF24, IHF23, and IHF24) and two for FS (TN23 and TN24) (Table S1 in Online Resource 2). Observations for rust severity at parental-level did not deviate significantly from normality ($W = 0.981$, $p = 0.54$), whereas significant departures were detected at the family ($p = 0.027$) and individual levels ($p < 10^{-37}$) (Table S2, in Online Resource 2, Fig. S2 in Online Resource 1). The distribution pattern of BLS severity showed no significant deviation from normality ($p > 0.10$) at parental- and family-levels, but

238 individual-level observations showed strong non-normality ($W = 0.85$, $p < 10^{-55}$) (Table S2 in
239 Online Resource 2, Fig. S3 in Online Resource 1). In contrast, FS exhibited substantial
240 deviations from normality at all levels, including in parental, family, and individual datasets ($p <$
241 10^{-6}) (Table S2 in Online Resource 2, Fig. S4 in Online Resource 1).

242 Rust severity was highest at IHF and TN, where scores were predominantly moderate to
243 high (4–7) in both years (Fig. 1). At TIF in 2023, rust symptoms were visible, but high BLS
244 severity interfered with accurate phenotyping; therefore, rust was not scored that year. In 2024,
245 disease pressure for both rust and BLS allowed for concurrent scoring, with rust scores strongly
246 skewed toward resistance (0–1). BLS severity at IHF was low in 2023 (scores 0–1) before
247 increasing slightly in 2024 (scores 1–4). Conversely, TIF experienced heavy BLS severity in
248 2023, with scores peaking between 6 and 8, followed by a substantial decrease in 2024 to the 1–4
249 range. FS severity distributions at TN remained consistent across years, with 0 being the most
250 frequent score, followed by 5, 7, and 9 (Fig. 1).

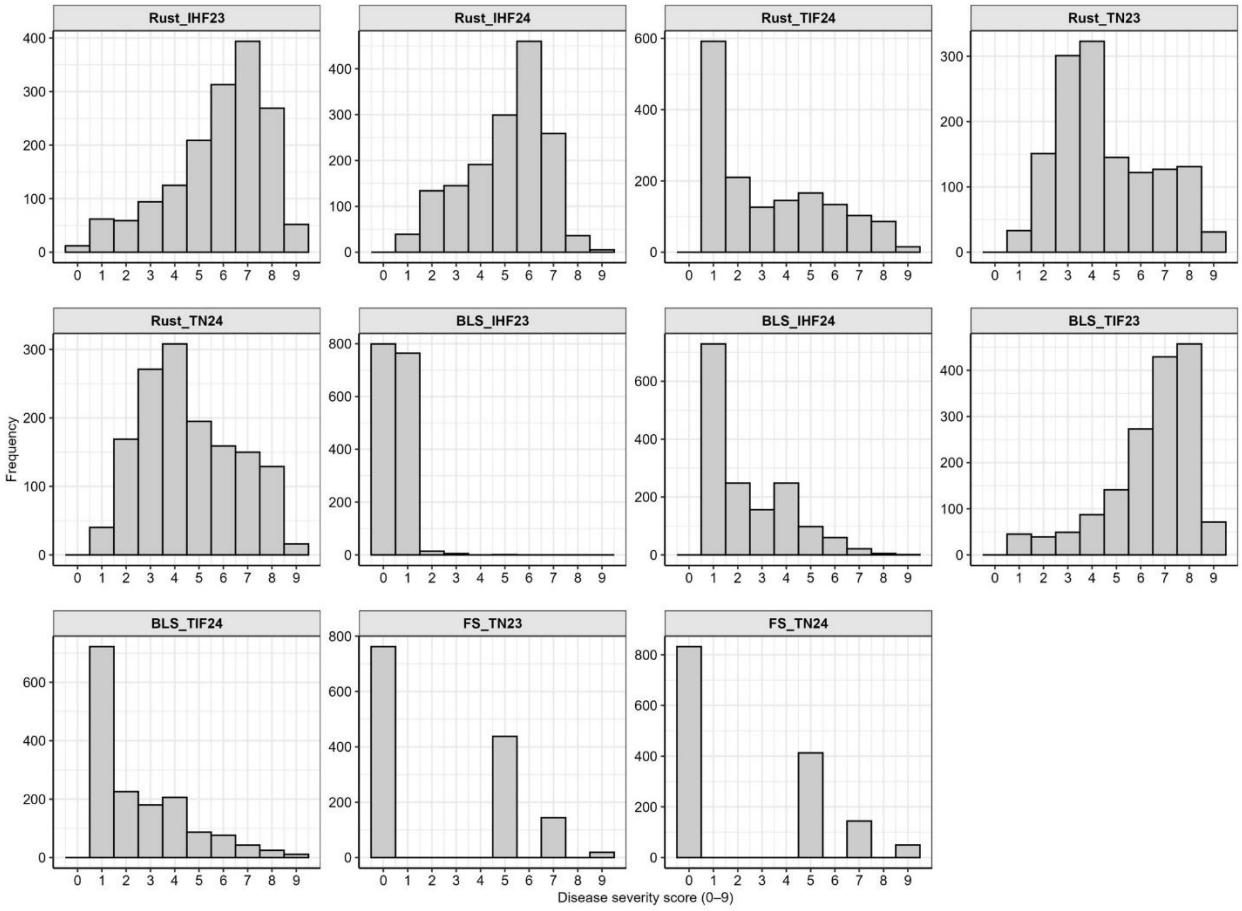


Fig. 1 Frequency distribution of the 800 switchgrass individuals (from 200 half-sib families) across the 0–9 severity scales for rust, Bipolaris leaf spot (BLS), and false smut (FS) evaluated in 2023 and 2024 at three locations (Ironhorse farm, Watkinsville, GA-IHF; Gibbs Farm, Tifton, GA-TIF; University of Tennessee-Knoxville, TN)

Variance component analysis and broad-sense heritability (H^2)

A linear mixed model was fitted to partition variance components and estimate H^2 across three hierarchical levels: parental, family, and individual. Shapiro–Wilk statistics were consistently higher ($W \approx 0.93–0.99$) across all traits and levels, indicating improved adherence to normality

relative to the raw data. However, the Shapiro–Wilk test detected statistically significant deviations from normality for all traits and levels ($p < 10^{-5}$) due to the large sample sizes. Despite this, Q-Q plots (Fig. S5 in Online Resource 1) showed residual distributions that closely followed the normal distribution.

High H^2 estimates were observed for rust (0.76–0.77) and FS (0.76–0.78), which remained consistent across all three levels of analysis (Table 1). For all diseases, the individual-level model captured the highest total genetic variance. For BLS, H^2 at the family-level (0.49) and clonal repeatability at the individual level (0.47) were notably higher than the parental-level estimate (0.44). This relative reduction in H^2 for BLS was driven by higher levels of genotype-by-environment interaction and residual variance relative to the genetic variance.

Table 1 Variance components and broad-sense heritability (H^2) for rust, Bipolaris leaf spot (BLS), and false smut (FS) resistance across parental, family and indvidual levels in a switchgrass population of 200 half-sib switchgrass families evaluated across three locations (Ironhorse farm, Watkinsville, GA-IHF; Gibbs Farm, Tifton, GA-TIF; University of Tennessee-Knoxville, TN) and two years (2023 and 2024)

Disease	Level	Genetic	$G \times E$	Residual	Env	Rep	H^2
Rust	Parental	1.07	0.45	2.56	5	2	0.76
Rust	Family	1.28	0.99	1.84	5	2	0.77
Rust	Individual	1.45	1.01	1.64	5	2	0.76
BLS	Parental	0.24	0.27	1.89	4	2	0.44
BLS	Family	0.30	0.46	1.62	4	2	0.49
BLS	Individual	0.37	0.47	1.54	4	2	0.47
FS	Parental	4.32	0.59	3.86	2	2	0.77
FS	Family	4.33	0.57	3.68	2	2	0.78
FS	Individual	5.07	0.69	2.83	2	2	0.76

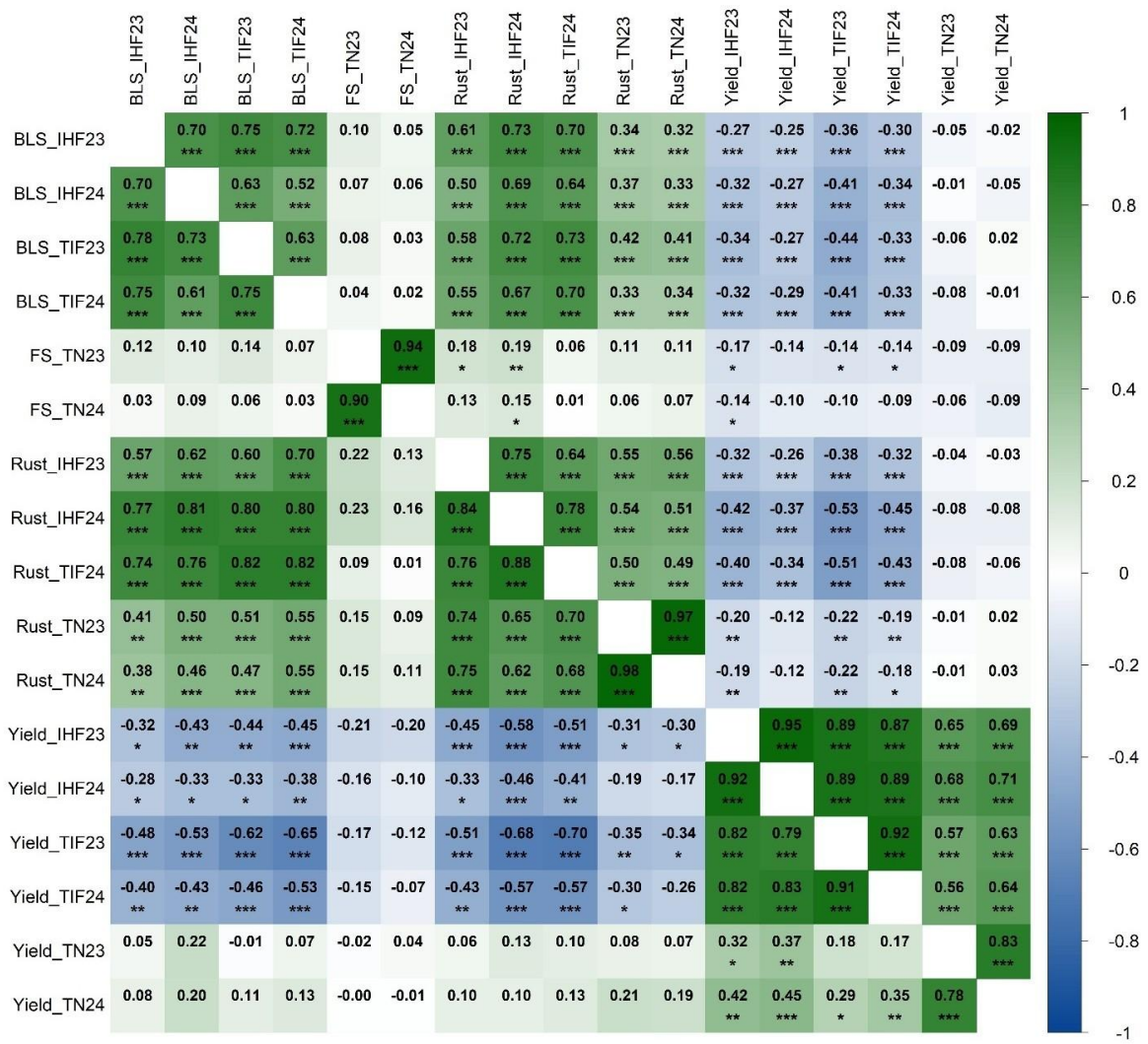
The variance components and heritabilities for rust and BLS were calculated across IHF and TIF, and for FS across IHF and TN using a linaer mixed model [19,38]; Env: environment representing location and year combination.

Correlations between disease and biomass yield

Pearson correlation coefficients were calculated to evaluate relationships between disease severities and biomass yield at both family-level and parental-level using family and parental BLUPs from the mixed model (Equation 1). Within and between environment correlation coefficients were positive and highly significant ($P<0.001$) for each disease. Correlation coefficients of rust severity between environments ranged from 0.49 to 0.97 at family-level (Fig.

2, upper triangle) and from 0.62 to 0.98 at parental-level (Fig. 2, lower triangle). Differences between family and parental correlations were observed at the Georgia locations; in contrast, correlations of rust severity between years in TN were very similar (r = 0.97 at family-level and r = 0.98 at parental-level). Correlation coefficients of BLS severity between environments ranged from 0.52 to 0.75 at the family-level and from 0.61 to 0.78 at the parental-level. Differences between family and parental correlations were observed across most environmental combinations, with the exception of IHF23 vs IHF24. Correlation coefficient of FS severity between TN23 and TN24 was 0.94 at the family-level and 0.90 at the parental-level.

Significant positive correlations were observed between rust and BLS within and between environments, with coefficients ranging from 0.32 to 0.73 at the family-level and 0.38 to 0.82 at the parental-level. Conversely, significant negative correlations were found between the two diseases (rust and BLS) and biomass yield. The correlation coefficients between biomass yield and rust reached as high as -0.53 (family-level) and -0.70 (parental-level), correlations between biomass yield and BLS reached -0.44 (family-level) and -0.65 (parental-level). Non-significant or weak correlations were observed between FS in TN environments and biomass yield across all environments, with coefficients ranging from -0.01 to -0.17 at the family-level and from 0.04 to -0.21 at the parental-level.



303

304 **Fig. 2** Correlation between disease severities (rust, Bipolaris leaf spot [BLS], and false smut
305 [FS]) and biomass yield [19] in switchgrass half-sib population across three locations (Ironhorse
306 Farm, Watkinsville, GA-IHF; Gibbs Farm, Tifton, GA-TIF; University of Tennessee-Knoxville,
307 TN) and two years (2023 and 2024); correlation analysis was performed at the family-level
308 (upper triangle: 200 half-sib families) and parental-level (lower triangle: 50 parents); color
309 intensity and numerical values indicate the Pearson correlation coefficient; significance levels are
310 indicated by asterisks: *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$

Multi-environment stability analysis

BLUP based selection of candidates for stability analysis

The list of the top 20 families selected for each disease includes eight (for rust), four (for BLS), and three (for FS) families that were also ranked in the top 20 for biomass yield by Irfan et al., [19]. The top 20 families for each disease represent a total of 14 unique parental accessions (Table S3 in Online Resource 2). The selected 20 families for rust represent 55% coastal, 30% lowland, and 15% unknown switchgrass ecotypes (Fig. S6 in Online Resource 1). Likewise, the selected 20 families for BLS represent 35% coastal, 50% lowland, and 15% unknown switchgrass ecotypes. The selected 20 families for FS represent 20% coastal, 70% lowland, and 10% unknown switchgrass ecotypes. Analysis of phenotypic performance (disease score) across the top 20 families revealed that all selected families exhibited negative deviation values (Fig. S7 in Online Resource 1), confirming their superior rust resistance relative to the population mean.

Three half-sib families: f116, f135 (progeny of J019.A), and f79 (progeny of B6) consistently outperformed the rest. Similarly, the top 20 families for BLS exhibited negative overall (Fig. S7 in Online Resource 1) and IHF-specific BLUP deviation values (Fig. S8 in Online Resource 1). The three families with the most pronounced negative overall BLUP deviations were f 196 (progeny of J312.A), f5 (progeny of J320.A), and f126 (progeny of J497.A). Performance analysis for FS was conducted exclusively for the TN location. In the other locations, although FS symptoms were observed, they were present in less than 1% of population Rijal et al., [unpublished] and were not scored. All top 20 selected families exhibited negative deviation values of FS severity, indicating high levels of resistance relative to the

population mean; f36 and f91 (progeny of J317.A), and f153 (progeny of J463.A) were the top performers.

AMMI analysis of variance

The AMMI ANOVA revealed highly significant effects of environment and genotype (family) on rust and BLS predicted severity values ($P < 0.001$; Table 2). Environments accounted for the largest proportion of the total sum of squares (92.6% for rust and 99.6% for BLS), indicating a strong influence of environmental conditions on disease severity. The genetic variability among selected families for rust and BLS is explained by approximately 7.3% (rust) and 0.4% (BLS) of the total variation. The G × E interaction component, although relatively small in magnitude, was statistically significant (Table 2). The interaction was effectively captured by the first two interaction principal component axes, which together explained 90.74% (rust) and 86.30% (BLS) of the interaction sum of squares (Fig. S9 in Online Resource 1).

The AMMI ANOVA for raw disease severity scores revealed that the environment was a significant source of variation for both rust ($P = 0.016$) and BLS ($P = 0.002$), accounting for 21.11% and 57.31% of the total variation, respectively (Table S5 in Online Resource 2). For rust, the effect of family, and the $G \times E$ were highly significant ($P < 0.001$), explaining 3.79% and 15.57% of the total variation. Although IPCA1 explained 57.4% of the interaction and IPCA2 explained 25.3% (Fig. 3), the F test was not significant (Table S5 in Online Resource 2). For BLS, non-significant effect of family ($P = 0.296$), and the $G \times E$ ($P = 0.411$) was observed. The interaction was primarily captured by the IPCA1 (76.7%) and IPCA2 (19.5%) (Fig. 3). When using raw severity data, a large proportion of the variation was attributed to residuals (52.38%

for rust and 34.62% for BLS). This high level of residual variation likely masked the interaction significance for BLS compared to previous analyses using BLUPs, where the error terms were minimized.

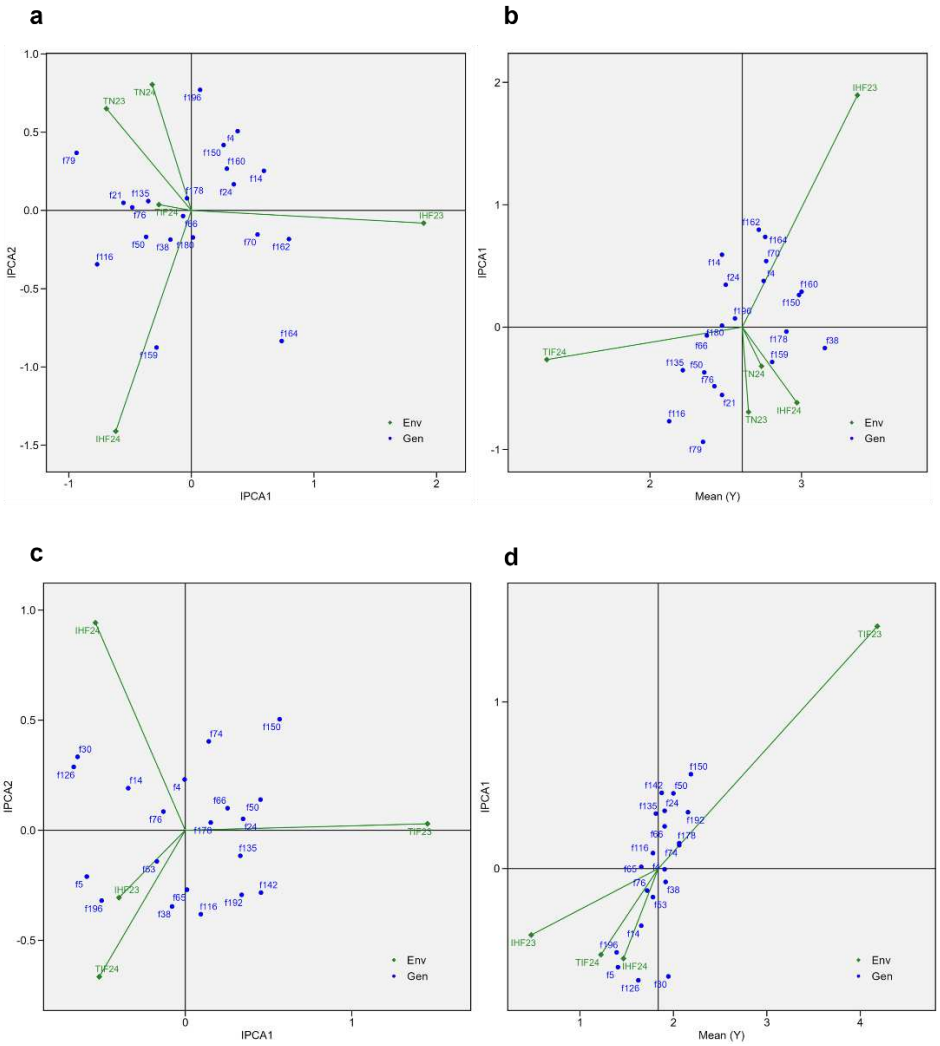


Fig. 3 Additive main effects and multiplicative interaction (AMMI) biplots for (a-b) rust and (c-d) Bipolaris leaf spot (BLS) severities; analysis was performed on top 20 switchgrass half-sib families selected for disease resistance across environments, including Ironhorse Farm, Watkinsville, GA (IHF); Gibbs Farm, Tifton, GA (TIF); and the University of Tennessee, Knoxville, TN (TN) during 2023 and 2024; the left panel shows the AMMI biplot of IPCA1 vs. IPCA2, accounting for of the G × E interaction; the right panels display the IPCA1 scores plotted against the mean severity scores; environment labels are shown in green, and family labels are shown in blue

Table 2 Additive main effects and multiplicative interaction (AMMI) analysis of variance based on best linear unbiased predictions (BLUPs) for rust and Bipolaris leaf spot severity; data represents the top 20 families across five environments for rust (IHF 2023, IHF 2024, TIF 2024, TN 2023, and TN 2024) and four environments for BLS (IHF2023, IHF 2024, TIF 2023, and TIF 2024)

Source of variation	Df	Sum of squares (SS)	Mean SS	F-value	P-value
Rust					
Genotypes	19	5.51	0.29	316.66	<0.001 ***
Environments	4	69.44	17.361	18956.98	<0.001***
Interactions	76	0.07	0.001		
IPCA 1	22	0.05	0.002	11.39	<0.001***
IPCA 2	20	0.02	0.001	4.14	<0.001***
Residuals	34	0.01	0		
Bipolaris leaf spot					
Genotypes	19	1.5	0.08	84.08	<0.001***
Environments	3	374.19	124.73	132888.7	<0.001***
Interactions	57	0.05	0		
IPCA 1	21	0.03	0	3.06	0.0114 *
IPCA 2	19	0.02	0	2.25	0.049 *
Residuals	17	0.01	0		

Significance levels are indicated by asterisks: *** P < 0.001 and * P <0.05.
Locations include Ironhorse Farm, Watkinsville, GA (IHF); Gibbs Farm, Tifton, GA (TIF); and the University of Tennessee, Knoxville, TN (TN).

Among the selected families, the mean severity and BLUP for rust and BLS differed markedly among environments (Fig. 3, Fig. S9 in Online Resource 1). The highest value was observed in IHF23, for rust and TIF23 for BLS, whereas TN24 and IHF23 have the lowest values for rust and BLS, respectively. These observations indicated that IHF had a more rust-conducive environment and Tifton had a more BLS-conducive environment. The interaction effects between environment and genotype differed in both magnitude and direction, as reflected by contrasting IPCA scores. Interaction patterns among families and environments were observed in the AMMI biplot (IPCA1 vs IPCA2) (Fig. 3, Fig. S9 in Online Resource 1). Families clustering near the origin exhibited minimal interaction effects and greater stability across environments. families with low mean disease severity and small absolute IPCA values were identified as broadly adapted and stable for disease resistance.

Selection using combined ranking

AMMI stability values (ASV) ranged from 0.17 to 0.42 for rust and from 0.13 to 0.19 for BLS (Table 3). The lowest rust ASV values were observed for f79 and f160, ranking them first for high stability across environments. Ten families shared the same minimum ASV value (0.13) for BLS, showing considerable stability. Higher ASV values indicated stronger interaction effects and greater sensitivity to environmental variation.

By integrating BLUP values for disease severity and stability across all environments, the CR for rust and BLS enabled the identification of families with superior, stable performance (Tables 3). For rust, Fam 116 ranked first based on CR, followed by Fam 135 and Fam 79. For BLS, Fam 196, Fam 5, and Fam 126 were ranked in the top three. Eight families were present in

both rust CR and BLS CR rankings, indicating broad-spectrum resistance to both diseases. These families with low overall rust severity and favorable stability make strong candidates for breeding advancement.

Table 3 Development of combined ranking method for rust (RCR) and Bipolaris leaf spot (BCR) by ranking switchgrass half-sib families based on environment-specific severity BLUPs and AMMI stability values (ASV); environments include Ironhorse Farm, Watkinsville, GA (IHF); Gibbs Farm, Tifton, GA (TIF); and the University of Tennessee, Knoxville, TN (TN) in 2023 and 2024; stability analysis was performed on the top 20 families selected based on their BLUP values for disease severities indicating higher resistance to diseases (low BLUP values)

Rust								Bipolaris leaf spot						
ID	Mean BLUP TN23	Mean BLUP TN24	mean BLUP TIF24	mean BLUP IHF23	Mean BLUP IHF24	ASV	RCR	ID	Mean BLUP IHF23	mean BLUP IHF24	mean BLUP TIF23	mean BLUP TIF24	ASV	BCR
J019.Af116	2.400[1]	2.500[1]	1.200[1]	3.700[1]	2.900[1]	0.418[4]	9[1]	J312.Af196	-0.502[1]	1.374[1]	5.430[1]	1.546[1]	0.193[4]	8[1]
J019.Af135	2.500[2]	2.500[1]	1.200[1]	3.800[2]	3.000[2]	0.292[3]	11[2]	J320.Af5	-0.501[2]	1.375[2]	5.431[2]	1.547[2]	0.193[4]	12[2]
B6f79	2.500[2]	2.600[2]	1.300[2]	3.900[3]	3.100[3]	0.167[1]	13[3]	J497.Af126	-0.360[3]	1.516[3]	5.572[3]	1.688[3]	0.132[1]	13[3]
B6f50	2.600[3]	2.700[3]	1.400[3]	3.900[3]	3.100[3]	0.418[4]	19[4]	J323.Af14	-0.340[4]	1.536[4]	5.592[4]	1.708[4]	0.167[2]	18[4]

J497.C f66	2.600[3]	2.700[3]	1.400[3]	3.900[3]	3.100[3]	0.418[4]	19[4]	J496.C f65	-0.340[4]	1.536[4]	5.592[4]	1.708[4]	0.167[2]	18[4]
J323.A f14	2.700[4]	2.700[3]	1.400[3]	4.000[4]	3.200[4]	0.292[3]	21[5]	J498.B f76	-0.300[5]	1.576[5]	5.632[5]	1.749[5]	0.193[4]	24[5]
J324.A f21	2.700[4]	2.700[3]	1.400[3]	4.000[4]	3.200[4]	0.292[3]	21[5]	J019.A f116	-0.259[6]	1.617[6]	5.673[6]	1.789[6]	0.132[1]	25[6]
J498.B f76	2.700[4]	2.700[3]	1.400[3]	4.000[4]	3.200[4]	0.292[3]	21[5]	J247.A f53	-0.259[6]	1.617[6]	5.673[6]	1.789[6]	0.132[1]	25[6]
J019.A f180	2.700[4]	2.800[4]	1.500[4]	4.000[4]	3.200[4]	0.418[4]	24[6]	J019.A f135	-0.239[7]	1.637[7]	5.693[7]	1.809[7]	0.167[2]	30[7]
B6 f24	2.700[4]	2.800[4]	1.500[4]	4.000[4]	3.200[4]	0.418[4]	24[6]	J497.C f142	-0.199[8]	1.677[8]	5.733[8]	1.850[8]	0.193[4]	36[8]
J312.A f196	2.800[5]	2.800[4]	1.500[4]	4.100[5]	3.300[5]	0.292[4]	26[7]	J498.B f38	-0.180[9]	1.696[9]	5.752[9]	1.868[9]	0.132[1]	37[9]
J497.C f162	2.900[6]	3.000[5]	1.700[5]	4.200[6]	3.400[6]	0.418[4]	32[9]	B6 f24	-0.179[10]	1.697[10]	5.753[10]	1.870[10]	0.132[1]	41[10]
J496.C f4	2.900[6]	3.000[5]	1.700[5]	4.200[6]	3.500[7]	0.284[2]	31[8]	J497.C f66	-0.179[10]	1.697[10]	5.753[10]	1.870[10]	0.132[1]	41[10]
J237.A f159	3.000[7]	3.000[5]	1.700[5]	4.300[7]	3.500[7]	0.292[3]	34[10]	J496.C f4	-0.179[11]	1.697[10]	5.753[10]	1.870[10]	0.132[1]	42[11]

J319.A f164	3.000[7]	3.000[5]	1.700[5]	4.300[7]	3.500[7]	0.292[3]	34[10]	J320.A f30	-0.164[12]	1.712[11]	5.768[11]	1.885[11]	0.132[1]	46[12]
J499.A f70	3.000[7]	3.000[5]	1.700[5]	4.300[7]	3.500[7]	0.292[3]	34[10]	B6 f50	-0.104[13]	1.772[12]	5.828[12]	1.944[12]	0.184[3]	52[13]
J041.A f178	3.100[8]	3.100[6]	1.800[6]	4.400[8]	3.600[8]	0.292[3]	39[11]	J041.A f178	-0.081[14]	1.795[13]	5.851[13]	1.967[13]	0.132[1]	54[14]
J463.A f150	3.100[8]	3.200[7]	1.900[7]	4.400[8]	3.700[9]	0.284[2]	41[12]	J215.A f74	-0.078[15]	1.798[14]	5.854[14]	1.971[14]	0.132[1]	58[15]
J326.A f160	3.100[8]	3.200[7]	1.900[7]	4.500[9]	3.700[9]	0.167[1]	41[12]	J321.A f192	-0.017[16]	1.859[15]	5.915[15]	2.031[15]	0.184[3]	64[16]
J498.B f38	3.300[9]	3.300[8]	2.000[8]	4.600[10]	3.800[10]	0.292[3]	48[13]	J463.A f150	0.003[17]	1.879[16]	5.935[16]	2.051[16]	0.193[4]	69[17]

405 ID: parent ID followed by family ID;
406 Numbers in superscript indicate respective rankings for resistance and stability; the selection indices (RSI and BSI) are summation of
407 ranks across environmental BLUPs and ASV.

Discussion

Sustainable biomass production systems require disease management strategies that reduce input costs and environmental impacts. The development of disease-resistant cultivars is an effective approach to achieving this objective. In this study, we evaluated a switchgrass half-sib population and, through phenotypic selection supported by multi-environment stability analysis, identified the top 20 families with resistance to major diseases in Georgia and Tennessee. The population was assessed for disease severity over two years (2023–2024) across three locations: IHF, TIF, and TN. Biomass yield of the same population was evaluated independently across these locations for three years (2022–2024) [19].

The population followed a hierarchical structure: 800 individuals from 200 half-sib families derived from 50 parental accessions. Trials were conducted at the same locations as a diversity panel of 331 switchgrass genotypes, which included the 50 parental accessions [25, 32, 33]. The diversity panel and several switchgrass populations have been studied under the Department of Energy (DOE) and the Center for Bioenergy Innovation (CBI) [33, 39]. These studies were conducted at the University of Georgia and the University of Tennessee Research Farms. These locations have historically shown natural diseases pressure for rust [40, 41], BLS [42, 43], and FS [10], facilitating the disease phenotyping and the selection of resistant genotypes.

Disease expression is highly sensitive to environmental conditions, and effective management requires an understanding of how location-year combinations influence both pathogen pressure and host response [15, 40]. In our study, only TN environments were conducive to FS severity. Although there have been no prior published reports of FS in

Tennessee, it was documented in our previous study in Georgia since 2019 [10]. While FS incidence was comparatively higher in the diversity panel established at IHF, the half-sib population did not exhibit similarly high incidence and severity for data scoring Rijal et al., [unpublished].

The TIF environments were more conducive to BLS and rust, whereas the IHF environments were primarily conducive to rust. These patterns align with our observations that BLS is favored by the warm, humid conditions typical of South Georgia summers, whereas rust and FS thrive under cooler, humid conditions during the fall season in both GA and TN [44]. Although BLS in switchgrass has been reported in Tennessee since 2007 [42], the populations evaluated in this study showed low BLS incidence; therefore, severity data were not collected in TN. Rust has been reported to infect switchgrass in TN and GA since 2007 and 2011, respectively [41, 45].

Rust severity showed high correlation within GA locations across years (0.64-0.78) and intra-location correlation in TN (0.97), suggesting similarity in races across years and overwintering of the inoculum, which serves as the primary inoculum in the next growing season. The significant correlations (0.49-0.56) in rust severity between GA and TN suggest a similar pathogen inoculum across these regions, providing support for the regional migration of rust inoculum previously proposed by Rijal et al., [unpublished]. Similar trend of rust inoculum migration is documented in wheat rust across the U.S. [46]. Consistent with this, population genetic studies of rust have identified two distinct clusters corresponding to northern and southern U.S. isolates [15, 40]. Correlations of BLS severity between locations and years, and FS severity within TN across years remained consistently significant and high. Collectively, these strong correlations suggest that the pathogen populations remained stable across the studied

453 years and locations, ensuring that host resistance rankings remain predictable even under varying
454 levels of environmental pressure.

455 The family-level H^2 for rust, BLS, and FS were 0.77, 0.49, and 0.78, respectively. The
456 higher broad-sense heritability (H^2) estimates for rust and FS suggest that phenotypic differences
457 among families are driven by genetic factors, and that selection for resistance to these diseases
458 should be effective. In contrast, the moderate H^2 for the BLS severity and biomass yield (H^2 =
459 0.50) [19] implies a relatively greater contribution of environmental and residual variation.
460 Higher H^2 indicates strong potential for genetic improvement through recurrent selection [47].
461 There are currently limited studies dissecting the heritability of disease resistance in switchgrass.
462 Consistent with our study, H^2 for rust in half-sib families of switchgrass has been reported to be
463 up to 0.73 [48] Likewise, the H^2 for maize rust caused by *Puccinia sorghi* ranged from 0.83 to
464 0.93 [49], and wheat rust caused by *Puccinia striiformis* f. sp. *tritici* was 0.86 [50]. The moderate
465 H^2 in our study is consistent with a GWAS for BLS resistance in a northern switchgrass
466 association panel, where the H^2 estimates ranged from 0.32 to 0.61 [12]. To our knowledge, no
467 previous studies have specifically characterized the heritability of FS severity in switchgrass.

468 Strong positive correlation between BLS and rust disease severity was observed in this
469 study, while the correlations with FS were negligible. It is worth noting that, unlike rust and
470 BLS, which infect leaves and stems, FS symptoms are observed in reproductive parts. The
471 biomass yield data [19] from all six environments were incorporated, allowing us to identify
472 correlations between disease severity and biomass yield. Although FS may not directly affect
473 biomass production, indicated by non-significant correlation, it can significantly impact seed
474 production and switchgrass propagation, making it an important trait to consider in breeding
475 programs. Because FS severity and biomass were not correlated in this study, biomass

performance must be considered independently when selecting for FS resistance. On the other hand, the notable negative correlation observed between rust/BLS severities and biomass yield in our study suggests that selecting for resistance removes constraint on biomass potential. This trend is seen in other crops, where negative correlation between rust severity and grain yield is reported, including maize (-0.3) [49], soybean (-0.6) [51], and wheat (-0.58) [52]. Integrating multi-environment disease phenotyping with biomass performance is therefore critical for improving resistance without compromising yield, a central objective for bioenergy crop breeding.

Underlying genetic control of rust, BLS, and FS resistance in switchgrass is not yet fully understood; both qualitative and quantitative components may contribute to field resistance. A handful of studies have identified quantitative trait locus (QTL) or marker associations for rust and BLS resistance in switchgrass, indicating that at least part of the variation is genetically tractable, but application of identified loci in improving resistance in breeding lines has yet to be verified [8, 12]. In this context, phenotypic recurrent selection at the family level remains a practical and effective strategy for simultaneously improving disease resistance and biomass yield [18, 53]. Accordingly, we based our primary selection decisions on family-mean performance and stability, using mixed-model BLUPs and multi-environment trial data. Family-level analysis was used for selection decisions on biomass yield in our prior study [19]. Using severity BLUPs from the mixed model, we ranked families and selected the top 20 for each disease, identifying three families with multi-disease resistance and eight that were resistant to both rust and BLS. Among the selected families, eight (for rust), four (for BLS), and three (for FS) families are the top biomass-yielding families previously identified [19], indicating that simultaneous improvement of resistance and biomass is feasible. These families provide an

immediate pool of elite candidates for advancing subsequent selection cycles or for use as parents in new crosses to stack resistance while retaining high biomass potential.

Although the overall performance of the selected families indicates significant genetic potential, environmental stability testing remains imperative for identifying consistently resistant genotypes. We further assessed the environmental stability of the 20 half-sib families selected for rust and BLS resistance using AMMI-based analysis. The dominant contribution of the environment to total variation (92.6% for rust and 99.6% for BLS) highlights the profound influence of location-year combinations on disease severity. For complex traits with high G × E, multi-environment testing is necessary to make selection decisions [20, 53]. In this study, the application of BLUP-based AMMI analysis proved more robust than the analysis using raw phenotypic means. When using raw data, the residual effect remained high, effectively masking the G × E interaction. The shrinkage property of BLUPs provided a reliable estimate of the interaction stability [21]. Our results confirm that for unbalanced designs or trials with high environmental pressure, using BLUPs as input for AMMI provides a more precise biological interpretation of stability than raw phenotypic data.

Within the selected families, the contribution of G × E interaction to total variation was significant but relatively small (0.09% for rust and 0.01% for BLS). The significant IPCA1 and IPCA2 axes indicate consistent differential responses of families across environments. Families such as f116 and f135 (progeny of J019.A), and f79 (progeny of B6) consistently combined low rust severity with favorable stability (ASV). Similarly, f196 (progeny of J312.A), f5 (progeny of J497.A), and f126 (progeny of J323.A) consistently combined low BLS severity with favorable ASV. AMMI analysis has been used for resistance to diseases in crops like mungbean (for leaf spot [29], rice (for blast [54]), and wheat (for powdery mildew and yellow rust [55]) to

differentiate between germplasm for location-specific resistance deployment and across-locations deployment. In our study, families derived from J019.A, J312.A, and J498.B represent the most robust sources of combined resistance and stability (Table 3). Some families also exhibited location-specific resistance. For example, f21 and f164 (progeny of J324.A and J319.A) are relatively better for resistance to rust and BLS in TN environments than in GA. In contrast, f4 (progeny of J496.C) is better for resistance to rust and f150 (progeny of 463.A) for BLS resistance in GA environments than in TN. Conversely, we also identified families with high resistance but low stability for BLS, including f65 (progeny of J496.C) and f74 (progeny of J215.A).

In our selection, resistance to all diseases predominantly originated from accessions collected from Texas (50% for rust, 36% for BLS, and 36% for FS). Resistance to rust and BLS originated on average 32% from Louisiana and 7% from Florida, while FS resistance originated 7% each from Florida, Louisiana, Kansas, and Coahuila. Accessions from lowland and coastal ecotypes likely evolved to possess broad-spectrum disease resistance, in consistence with the findings that switchgrass are locally adapted to withstand biotic and abiotic conditions [15]. In contrast, FS-resistant accessions showed a more fragmented geographic distribution. It is likely that FS infection is localized and environment-dependent, causing selection pressure leading to region-specific resistance adaptation. Because infection occurs in reproductive tissues, host phenological traits such as flowering time may further influence host–pathogen interactions and contribute to host-specific specialization. This is consistent with observations in rice false smut caused by *Ustilaginoidea virens*, where infection is dependent on floral organs, particularly the presence of stamens [56].

The selection of disease-resistant genotypes can contribute to sustainable disease management; however, deploying resistant genotypes can impose strong selection pressure on pathogen populations [57]. Over time, this pressure may favor the emergence of virulent races capable of overcoming resistance, particularly when the deployed resistance is genetically narrow. Consequently, incorporating a genetic base with multiple resistance alleles while maintaining yield potential is essential for durable resistance [58]. Our selection pool can address this, where we have maintained 14 different parental lineages, thereby preserving allelic diversity that can buffer against pathogen adaptation and help sustain resistance over multiple deployment cycles.

Conclusion

This study presents an integrated framework for improving disease resistance in switchgrass by combining mixed-model BLUPs with AMMI-based stability analysis. The integration of mean performance and stability metrics provides a robust approach for identifying families with durable resistance and broad adaptation. The multi-disease-resistant and stable families identified here form a strong foundation for the next cycle of phenotypic recurrent selection and for the development of synthetic varieties tailored to southeastern U.S. production systems. Furthermore, the multi-environment phenotypic dataset generated in this study represents a valuable resource for future genomic selection and association mapping efforts aimed at enhancing durable resistance to rust, BLS, and FS in switchgrass.

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Statements & Declarations

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Competing Interests

Authors declare no conflict of interest.

Author Contributions

Samikshya Rijal: Conceptualization, Data curation, Formal analysis, Visualization, Validation, Project administration, Writing – original draft. **Shiva O. Makaju**: Formal analysis, Visualization, Validation, Writing – review & editing. **Mitra Mazarei**: Data curation, Formal analysis, Investigation, Project administration, Resources, Validation, Writing - review & editing. **Jazib A. Irfan**: Data curation, Writing – review & editing. **C. Neal Stewart**: Formal analysis, Investigation, Project administration, Resources, Supervision, Validation, Writing - review & editing. **Ali M. Missaoui**: Project administration, Resources, Supervision, Validation, Writing - review & editing, Funding acquisition. **James W. Buck**: Resources, Supervision, Validation, Visualization, Writing - review & editing. **Bochra A. Bhari**: Formal analysis, Investigation,

Project administration, Resources, Supervision, Validation, Visualization, Writing - review & editing, Funding acquisition.

Data availability

All data supporting the findings of this study are available within the supplementary materials.

Supplementary Information

Online Resource 1 (ESM_1.docx)

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Online Resource 2 (ESM_2. Xlsx)

This spreadsheet contains Tables S1-S6 which includes supporting materials and methods, and results.

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