Received: 21 November 2023 | Accepted: 23 March 2024

DOI: 10.1002/tpg2.20452

The Plant Genome

ORIGINAL ARTICLE

Globally deployed sorghum aphid resistance gene *RMES1* is vulnerable to biotype shifts but is bolstered by *RMES2*

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Assigned to Associate Editor Xianran Li.

Funding information
United States Agency for International Development, Grant/Award Number: AID-OAA-A-13-00047

Abstract

Durable host plant resistance (HPR) to insect pests is critical for sustainable agriculture. Natural variation exists for aphid HPR in sorghum (*Sorghum bicolor*), but the genetic architecture and phenotype have not been clarified and characterized for most sources. In order to assess the current threat of a sorghum aphid (*Melanaphis sorghi*) biotype shift, we characterized the phenotype of *Resistance to Melanaphis sorghi 1* (*RMES1*) and additional HPR architecture in globally admixed populations selected under severe sorghum aphid infestation in Haiti. We found *RMES1* reduces sorghum aphid fecundity but not bird cherry-oat aphid (*Rhopalosiphum padi*) fecundity, suggesting a discriminant HPR response typical of gene-for-gene interaction. A second resistant gene, *Resistance to Melanaphis sorghi 2* (*RMES2*), was more frequent than *RMES1* resistant alleles in landraces and historic breeding lines. *RMES2* contributes early and mid-season aphid resistance in a segregating F_2 population; however, *RMES1* was only significant with mid-season fitness. In a fixed population with high sorghum aphid resistance, *RMES1* and *RMES2* were selected for demonstrating a lack of severe antagonistic pleiotropy. Associations with resistance colocated with cyanogenic glucoside biosynthesis genes support additional HPR sources. Globally, therefore, an HPR source vulnerable to biotype shift via selection pressure (*RMES1*) is bolstered by a second common source of resistance in breeding programs (*RMES2*), which may be staving off a biotype shift and is critical for sustainable sorghum production.

Plain Language Summary

Global sorghum production is threatened by herbivorous insects and, recently, sorghum aphids. We tested hypotheses regarding global host plant resistance (HPR)

Abbreviations: BAP, Bioenergy Association Panel; Chr, chromosome; GBS, genotype-by-sequencing; GLM, general linear model; HCN, hydrogen cyanide; HPR, host plant resistance; KASP, kompetitive allele-specific polymerase chain reaction; LD, linkage disequilibrium; NIL, near-isogenic line; NLR, nucleotide-binding leucine-rich repeat; QTL, quantitative trait locus; RMES1, Resistance to *Melanaphis sorghi* 1; RMES2, Resistance to *Melanaphis sorghi* 2; SAP, Sorghum Association Panel; SNP, single nucleotide polymorphism.

and its risk of being overcome by emergent aphid populations. Sources of sorghum aphid HPR are limited and largely uncharacterized, with a few hypothesized mechanisms proposed. Using greenhouse and field aphid experiments combined with genetic data, we established the selection pressure of the globally used HPR source (R*esistance to Melanaphis sorghi 1* [*RMES1*]) on aphid populations and the availability of additional HPR sources providing resistance in wild and breeding genotypes. Responding to injurious cereal aphid biotype shifts has historically been slow and economically costly. A proactive genotype-to-phenotype map of *RMES1* and additional sources for HPR durability will inform cereal-aphid breeding efforts and address a major threat to global agricultural resilience.

1 | INTRODUCTION

Plant breeding indirectly affects insect populations by applying selection pressure via deployed host plant resistance (HPR). It is important for breeders to consider what HPR is being deployed in order to reduce the likelihood of population shifts in agronomically important pests or their emergence. Insect populations have regularly overcome HPR with genetic or geographical shifts into open niches (biotype shifts). For example, agronomically important biotypes of Russian wheat aphid (*Diuraphis noxia*) and greenbug (*Schizaphis graminum*) have shifted in the Great Plains in the 20th century, which resulted in severe yield loss or costly pesticide intervention (Harris-Shultz et al., 2020). Biotypic variation of soybean aphid (*Aphis glycines*) adapted to soybean HPR was proposed to have existed prior to commercial deployment or rapidly adapted (Yates & Michel, 2018). The *Nr* gene in lettuce and the *Ag1* resistance allele in raspberry were overcome by resistance-breaking aphid biotypes of the lettuce root aphid (*Pemphigus bursarius*) and large raspberry aphid (*Amphorophora agathonica*), respectively, in Europe (Keep, 1989; van der Arendt et al., 1999). Climate change is expected to exacerbate this problem as climate change–induced hybridization and habitable range expansion drive genetic diversity (Arce-Valdés & Sánchez-Guillén, 2022).

Aphids are economically significant pests, which remove photoassimilates, vector viruses, and can cause total yield loss. Plants have multiple layers of HPR against aphids including morphological barriers and chemical compositions, which can prevent feeding and infestation by deterring aphid behavior (antixenosis) and/or reducing fecundity (antibiosis) (Nalam et al., 2019). In contrast, tolerance is another type of plant response that allows the plant to maintain fitness under moderate infestation (Painter, 1951). While plant response to aphid infestation has been studied in model systems, our understanding of the molecular mechanisms behind aphid HPR in crops is limited (Nalam et al., 2019). In one example of monogenic constitutive antixenosis, sorghum aphid (*Melanaphis sorghi*; Theobald, 1904) feeding preference was affected in choice-assays by a *bloomless* gene knockout, which lacked cuticular wax while reproduction in no-choice assays did not (Cardona et al., 2022). This trait may be background dependent as a consistent effect on aphid damage was not observed across several different mutant genotypes (Harris-Shultz et al., 2019). A contrasting example of HPR is the monogenic induced antibiosis phenotype of the cloned *Mi-1* and *Vat* genes encoding nucleotide binding leucine-rich repeat (NLR) receptors (Dogimont et al., 2014; Nombela et al., 2003). Translational evidence from plant-pathogen research suggests that a gene-for-gene arms race could occur in plant–aphid systems, since resistance breaking biotypes of *Mi-1* and *Vat* have been identified and is one hypothesis for the boom-and-bust cycles of greenbug biotype-specific HPR (Dogimont et al., 2010; Kaloshian, 2004).

Sorghum [*Sorghum bicolor* L. (Moench)] is the world's fifth most important cereal and a staple crop for small-holder farmers in semiarid regions (FAOSTAT, 2022). Greenbug had historically been the primary agriculturally relevant aphid pest of sorghum in North America until a severe sorghum aphid outbreak occurred in the Americas beginning in 2013, which is believed to be due to a range expansion, and dispersed to all production regions within 5 years of its introduction (Armstrong et al., 2015; Harris-Shultz et al., 2020; Nibouche et al., 2021). Sorghum aphid primarily reproduces asexually in North America, where it exists as a super-clone and was recently distinguished from the sugarcane aphid (*Melanaphis sacchari*) by host preference as well as morphometric and molecular discrimination (Nibouche et al., 2021). A major HPR source to sorghum aphids is the globally deployed *Resistance to Melanaphis sorghi 1* (*RMES1*) on chromosome 6 (Chr06) found in African landraces where sorghum aphid was first described (Muleta et al., 2022; Vuillet, 1914). Whether the *RMES1* locus harbors one or more loci is undetermined (Cuevas et al., 2022). *RMES1* is rare in global sorghum landraces; however, it is expected to be common in

breeding programs and was swept to fixation in a recently established Haitian breeding population founded on globally admixed genotypes, capturing wide diversity, from East and West Africa (Muleta et al., 2022). The molecular mechanism of *RMES1* remains unknown. A second sorghum aphid HPR quantitative trait locus (QTL; *Resistance to Melanaphis sorghi 2* [*RMES2*]) co-localized with a transcription factor (*SbWRKY86*) whose functional allele may act as a regulatory hub for induced defenses (Poosapati et al., 2022). The prevalence and utility of this second sorghum aphid HPR source for breeding programs has not been established. In addition to cuticular waxes, other phytochemical herbivore deterrents, such as the cyanogenic glucoside dhurrin, have been proposed to contribute HPR, but variation has not been observed (Poulton, 1990; Woodhead et al., 1980).

The durability of HPR to aphid populations is expected to vary between tolerance and resistance as they apply different selection pressures, but study systems to test this are not tractable (Peterson et al., 2017). The Russian wheat aphid resistance gene *Dn4* was characterized as tolerant and resistant but was overcome by a biotype shift in western Colorado in 2003 (Haley et al., 2004; Hawley et al., 2003). The source of greenbug biotype C resistance in sorghum was considered as having tolerance but was overcome by biotype E shortly after wide deployment (Hackerott et al., 1969). A biotype C and E resistant grain sorghum breeding line, RTx2783, was identified as having tolerance, antixenosis-resistance, and antibiosis-resistance to sorghum aphids (Armstrong et al., 2015, 2017). RTx2783 contains the resistant *RMES1* allele and was a donor for sorghum aphid resistance in many cultivars grown on the Great Plains (Muleta et al., 2022). As RTx2783 may contain non-*RMES1* HPR sources, hypotheses on whether *RMES1* provides tolerance or resistance to sorghum aphid, and whether it provides resistance to other *Aphididae* species, have not been tested but would provide insight on its mechanism, durability, and the likelihood of biotype shifts.

Sorghum aphid and *S. bicolor* is an agronomically relevant system to study HPR and pest population dynamics (Zapata et al., 2018). There are competing hypotheses on whether *RMES1* provides resistance (antibiosis or antixenosis) or tolerance and whether that phenotype extends to other aphid species with implications for breeding. The contribution of *RMES2* to global breeding is unknown and molecular breeding tools have not been developed (Poosapati et al., 2022). It is also unclear whether *RMES1* and *RMES2* act additively or are epistatic. Here, we define the sorghum aphid-discriminant resistant phenotype of *RMES1* and demonstrate the potential for *RMES2* to bolster *RMES1* resistance based on global germplasm. Finally, we confirm the contribution of both HPR sources in a breeding program where *RMES2* is being selected alongside *RMES1*.

Core Ideas

- We investigated global sorghum aphid host plant resistance (HPR) and the risk of being overcome by biotype shifts.
- Selection pressure from the globally used *RMES1* on aphid populations was established using near-isogenic lines.
- *Resistance to Melanaphis sorghi* 2 (*RMES2*) contributes resistance in global diversity panels and is frequent in foundational US sorghum lines.
- *Resistance to Melanaphis sorghi 1* (*RMES1*) and *RMES2* contribute HPR in an F_2 population of globally admixed sorghum lines.
- *RMES2* is selected in a breeding program and likely contributes to global HPR, bolstering durability.

2 | MATERIALS AND METHODS

2.1 | Near-isogenic line development

RMES1 near-isogenic lines (NILs) were developed with a donor parent IRAT204 (sorghum aphid resistant, *RMES1* donor) and recurrent backcrossing to RTx430 (sorghum aphid susceptible; Muleta et al., 2022). Single plant selections were made at the F_2 stage of each (back)cross using a KASP (kompetitive allele-specific polymerase chain reaction) marker for *RMES1* (Sbv3.1_06_2892438R, Muleta et al., 2022), and homozygous plants were backcrossed to RTx430. BC_2F_3 progeny, hereafter referred to as NIL+ or containing resistant *RMES1* allele, and NIL− or containing susceptible *RMES1* allele, and parental genotypes were used for aphid bioassays and whole genome resequencing.

2.2 | Whole genome resequencing of NILs

Genomic DNA was collected from leaf tissue of BC_2F_3 NIL+, NIL−, IRAT204, and RTx430. Four plants of each genotype were grown, and DNA was extracted with Zymo Plant DNA Isolation Kits and sequenced individually. Samples were sequenced at the Genomics Shared Resource Core at the University of Colorado Anschutz Medical Campus. Raw reads were trimmed using trimmomatic v0.39 and mapped to the RTx430v2 reference (https://phytozome-next.jgi.doe.gov/info/SbicolorRTx430_v2_1) with BWA (Burrow's-Wheeler Aligner) v0.7.17-r1188 (H. Li & Durbin, 2009). Duplicate reads were identified using Picard v2.26 (http://broadinstitute.github.io/picard) (McKenna et al., 2010). Finally, variants were called using GATK v4.2.5.0.

2.3 | Aphid cultures

Sorghum aphids (*M. sorghi*) used in this study were received from Dr. Scott Armstrong at the USDA-ARS, Stillwater, OK. Aphids were reared on Tx7000 seedlings under laboratory conditions as previously described (Nalam et al., 2021). Seedlings were grown in 4.5″ pots with potting soil and top layer of sand to reduce damping off at a temperature of 24 ± 1˚C and a photoperiod of 16-h/8-h light/dark period. Colonies were housed in cages covered with insect-proof cloth. Bird cherry-oat aphid (*Rhopalosiphum padi*) cultures were collected from the Colorado State University greenhouses and maintained on Tx7000 seedlings similar to sorghum aphid cultures.

2.4 | No-choice assay for aphid fecundity

No-choice assays were used to compare aphid fecundity on various genotypes. A single seedling was grown in 6″ pots using potting soil and a top layer of greens grade (Profile Products LLC). At 3–4 weeks of age, or 2–3 leaf stage (GS1, Roozeboom & Prasad, 2019), three 3- to 4-day-old apterous sorghum aphids were placed at the base of the seedlings with a camel hairbrush. A clear plastic cylinder was placed over the plant to prevent aphids from leaving the pot with an organdy cloth covering for ventilation. The number of aphids on each plant was counted daily for a week at ∼12 p.m. A similar no-choice assay with bird cherry-oat aphid was used to determine cross-resistance. NIL+ and NIL− lines were infested with three 4- to 5-day-old apterous aphids and counted daily for 1 week and at 2 weeks after infestation. For sorghum aphid and bird cherry-oat aphid no-choice experiments, pots were randomized to reduce environmental effects and the final N reported reflects two replicated experiments. Student's *t*-test was used to determine fecundity differences between NIL genotypes in R v4.2.2.

2.5 | Choice assay for aphid settling preference

A choice assay was done to determine aphid settling preference at the seedling stage. A pairwise comparison was done between the NIL+ and NIL− genotypes. Seedlings of each genotype were grown approximately 2″ apart in 1 gallon pots using potting soil and a top layer of greens grade. Seedlings were thinned to one plant of each genotype per pot. At 3–4 weeks of age, or 2–3 leaf stage (GS1), twenty 3- to 4-day-old apterous sorghum aphids were placed in the center of a paper bridge between the seedlings. A clear plastic cylinder was placed over the plants to prevent aphids from leaving the pot with an organdy cloth covering for ventilation. The number of aphids on each plant was counted at 6, 12, 24, and 48 h post infestation (hpi). Pots were randomized to reduce environmental effects. The experiment was repeated for a total of 16 pots (experimental units). Student's *t*-test was used to determine settling preference differences between NIL genotypes at each timepoint in R v4.2.2. The overall effect of *RMES1* on aphid settling was tested using a two-way repeated measures ANOVA (analysis of variance) with the rstatix package v0.7.2.

2.6 | Resequencing-based association study of community association panels

Whole genome resequencing for 665 sorghum genotypes from the sorghum association panel (SAP) and bioenergy association panel (BAP) was used for association analyses with previously published phenotype data (Poosapati et al., 2022). Raw reads for the SAP were retrieved from the European Nucleotide Archive (RJEB50066) (Boatwright et al., 2022). Raw reads for the BAP were retrieved from DRYAD (https://doi.org/10.5061/dryad.4b8gtht99) (LeBauer et al., 2020).

Raw reads for both association panels were mapped to BTx623 v5.1 (sorghumbase.org/Sorghum_bicolorv5) reference using BWA-mem (H. Li & Durbin, 2009). Samtools (H. Li et al., 2009) was used to select properly paired reads and sort, Picard v2.26 (http://broadinstitute.github.io/picard) was used to remove duplicate reads, Samtools was used to remove low quality reads ("-Q 30"), and VarScan (https://sourceforge.net/projects/varscan/) was used for variant calling. Variants were filtered using bcftools v1.15.1 commands "F_MISSING < 0.9" and "MAF > 0.01" (Danecek et al., 2021). Variants were imputed with BEAGLE v5.2 using default parameters (Browning et al., 2018). The principal components (PCs) of a random subset of 500,000 single nucleotide polymorphism (SNP) variants were used to estimate population structure computed by TASSEL 5.0 CLI -PrincipalComponentsPlugin function. A general linear model (GLM) was used to determine associations with the -FixedEffectLMPlugin using the first three PCs and normalized sorghum aphid phenotypes (Bradbury et al., 2007; Poosapati et al., 2022). A second association analysis was performed with the highest associated variant at *RMES2* (S09_61521444) included as a fixed-effect covariate. Manhattan plots were generated in base R (v4.2.2). A Bonferroni-corrected threshold of significance was applied.

The most recent reference genome version (BTx623v5) is used throughout this paper and coordinates refer to the v5 coordinate system unless otherwise noted. For example, S09_61433682 refers to the variant at 61,433,682 bp on Chr09 in the BTx623v5 genome, while S09_57630053.v3 represents a variant at 57,630,053 in BTx623v3, and

S06_3222901vRTx430 represents a variant at 3,222,901 on the RTx430v2.1 with a different coordinate system.

2.7 | Population genomic analyses of *RMES2* in landraces and early breeding germplasm

Unimputed allele calls from the resequenced lines for Chr06_3096975 and Chr09_61521444 for 268 sorghum accessions with longitude, latitude, and/or germplasm origin were used to determine allelic distributions for *RMES1* and *RMES2*. Metadata was collected from GeneSys (www.genesys-pgr.org/c/sorghum). Geographic distributions and pie charts were generated using r/ggplot2 (v3.4.1) (Wickam, 2016).

2.8 | Fixation analysis of globally admixed germplasm resistant to sorghum aphid

The fixation analysis used here was previously described (Muleta et al., 2022). Briefly, the Chibas sorghum breeding program was founded in 2013 using global germplasm and extensively intercrossed using the *ms3* sterility system. Each generation, 5%–10% of the population, is selected for grain yield potential, maturity, and height preferences of Haitian farmers. Selected females are pollinated by five randomly selected male lines (of those selected for generation advancement) and the next generation begins again. Low-input conditions, which approximate small-holder farmer growing practices, were used for population development allowing for heavy sorghum aphid infestation in year-round growing seasons with tropical environments. The resulting aphid-resistant admixed population ($N = 296$) was genotyped using genotype-by-sequencing (GBS) and compared to a separate set of accessions representing global diversity ($N = 767$) (Lasky et al., 2015; Morris et al., 2013). Pairwise SNP differentiation (F_{ST}) was determined between admixed breeding lines and global accessions, and outlier loci were detected based on the inferred distribution of neutral F_{ST}.

2.9 | Association analysis of globally admixed F_2 population segregating for aphid resistance

A second F_2 population was developed by the Chibas sorghum breeding program and used for genetic trait dissection. Lines from the globally admixed germplasm resistant to sorghum aphid (fixed for *RMES1*) were crossed to new diverse material in order to develop globally admixed F_2 lines segregating for aphid resistance phenotypes (Muleta et al., 2022). Phenotyping data were collected in spring–summer 2022 in Haiti under heavy sorghum aphid infestation. Susceptible checks had over 1000 aphids per plant, infesting all leaves, and covering one-third to half of leaf area. Fields were irrigated once a week and no pesticides or fertilizers were used. Plants were phenotyped for fitness (alive/dead) at flowering initiation (six- to seven-leaf) and booting (eight- to nine-leaf) growth stages.

Tissue was collected for genotyping approximately 1 month after planting and genotyped using Diverse Array Technology (Jaccoud et al., 2001). Sequencing was mapped to the BTx623v3.1 reference genome (McCormick et al., 2018). Marker positions discussed in the text were converted to the BTx623v5.1 coordinate system by BLAST (https://phytozome-next.jgi.doe.gov/) with 100 bp flanking segments. Data were processed in R package dartR, and markers that were monomorphic had <50% call rate or <90% repeatability were removed. Data were converted to VCF (variant call format) format using a custom R script and imputation was done using Beagle 5.4 (Browning et al., 2018). Data were converted to numeric representation of reference alleles (0,1,2). After filtering, there were 1172 individuals with 8195 markers.

Fitness was modeled as a binary phenotype using the generalized linear model (1) as follows:

$$Y_{ij} = \mathbf{Q} + m_j + \varepsilon_{ij} \quad (1)$$

where Y_i is the observed phenotype state for plant i (coded as 0 for dead and 1 for alive) and fit with a binomial logit function; G_i is the random effect of the ith plant distributed MVN (multivariate normal) $(0, \sigma^2_G)$, where σ^2_G is the genetic variance; ε_i is the residual error; $\mathbf{Q}$ is a matrix of n by two fixed effects accounting for population structure estimated from the first two PCs of the genome-wide SNP set; and m is the fixed effect of marker j. A custom script was used to run model (1) with AsremlR for each marker (Butler et al., 2017). A Bonferroni-corrected threshold of significance was applied.

3 | RESULTS

3.1 | Marker-assisted selection isolates *RMES1* in NILs

Testing hypotheses on specific HPR sources such as *RMES1* and its phenotype requires suitable germplasm, which isolates resistant and susceptible alleles such as NILs (Figure 1a). We therefore backcrossed *RMES1*-donor IRAT204 to the susceptible RTx430 and generated $BC_2F_{3\text{-}4}$ lines using a KASP marker to track the *RMES1* QTL (Muleta et al., 2022). NIL+ were lines fixed for the resistant *RMES1* allele, and NIL− were fixed for the susceptible *RMES1* allele. Whole-genome sequencing confirmed that the marker-assisted backcrossing successfully reduced the donor genome complement in NILs. There was 71.7% and 24.8% of the NIL+ genome fixed for the recurrent and donor parent genomes, respectively, and

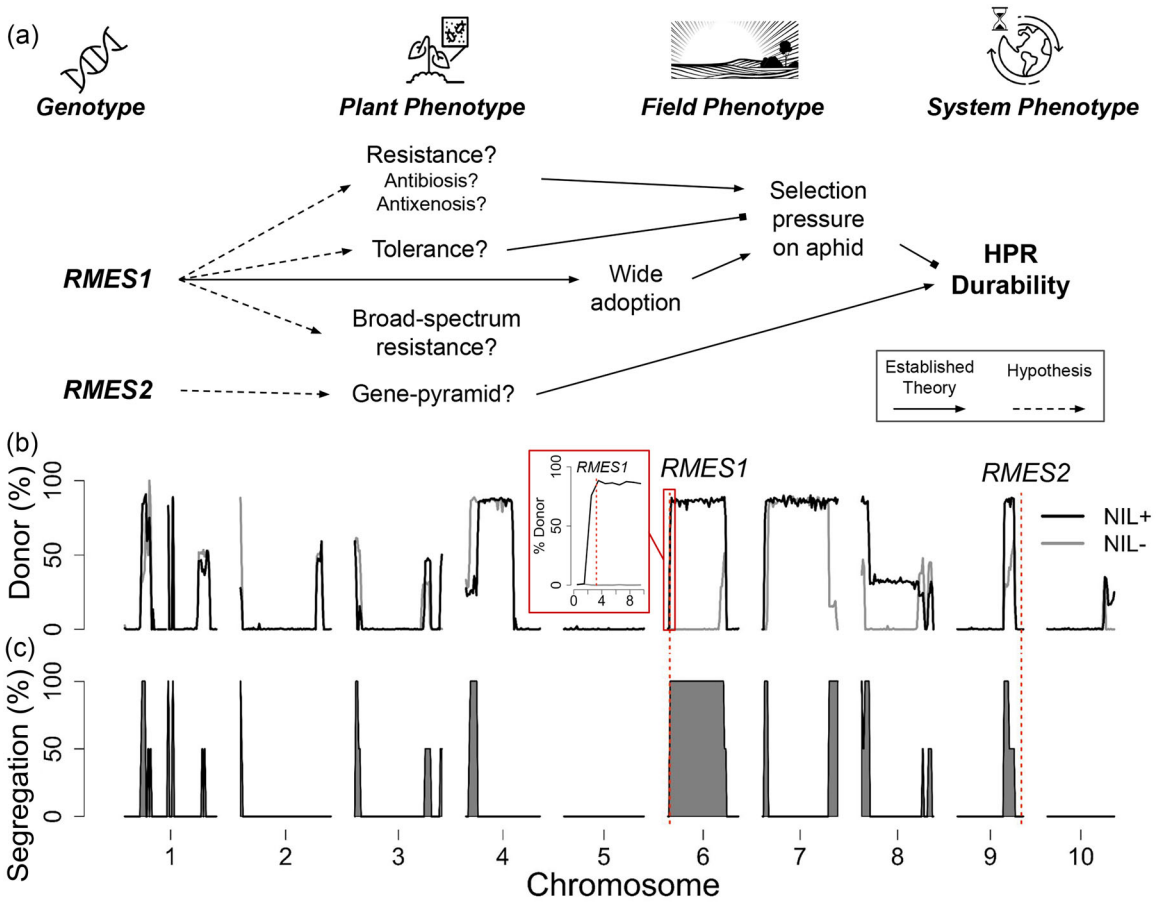

FIGURE 1 Genotype to phenotype hypotheses on *Resistance to Melanaphis sorghi 1* (*RMES1*) to be tested with near-isogenic lines. (a) The genotype to phenotype map for *RMES1*, *Resistance to Melanaphis sorghi 2* (*RMES2*), and host plant resistance (HPR) durability containing several established and hypothesized relationships. (b) Donor (IRAT204, 100%) genome contribution in BC_2F_3 near-isogenic lines (NILs) relative to recurrent genome (RTx430, 0%) using whole-genome sequencing (WGS) mapped to RTx430v2.1 reference genome. The inset panel is a close-up of the *RMES1* region on chromosome (Chr) 6. (c) Genomic regions segregating between NILs determined from WGS. Red dotted line indicates *RMES1* F_{ST}-identified single nucleotide polymorphism (SNP) on chromosome 6 (S06_2995581v3 = S06_3222901vRTx430) (Muleta et al., 2022) and the original *RMES2* GWAS-identified SNP on chromosome 9 (BTx623v3.1 S9_57630053, RTx430v2.1 S09_54801196) (Poosapati et al., 2022). Genomic analyses indicate that, as intended, NILs segregate for *RMES1* but not *RMES2*.

the remaining 3.4% was segregating (Figure 1b). The NIL− genome was 80.7% and 14.2% fixed for the recurrent and donor parent genomes, respectively, and 5.1% was segregated (Figure 1b). Comparing between NIL+ and NIL− sibling lines, 83.6% of the genome was isogenic and the remaining 16.3% was segregating, including the majority of Chr06 (Figure 1c). In the region of *RMES1* on Chr06, both NIL lines were fixed. However, there is a breakpoint in the NIL+ between 2,051,868 bp and 2,169,917 bp (RTx430v2 coordinates). The *RMES1* region, mapped in BTx623, corresponds to 2.9–3.1 Mb of the RTx430v2 genome and is, therefore, within the introgression. The previously reported *RMES2* SNP (S9_57630053v3) is monomorphic in IRAT204 and RTx430 as well as falling ~5 Mb outside of the introgressed region on Chr09. Therefore, these NILs allow us to test phenotypic hypotheses regarding *RMES1* without confounding sources of additional HPR.

3.2 | *RMES1* reduces fecundity of sorghum aphid

To determine whether antibiosis is a component of *RMES1*, we assessed aphid reproduction in a no-choice assay. Differences in population growth would indicate that the *RMES1* mechanism is retarding infestation and placing selection pressure on aphids. The number of aphids was lower on NIL+ than NIL− at 7 days post infestation (7 dpi) (Student's *t*-test, $p = 0.001$, $df = 15$; Figure 2a,b). An average difference of six aphids ($p = 0.025$) was seen at 3 dpi and 25 aphids ($p = 0.001$) at 6 dpi. The initial infestation of three aphids grew to an average of 28 ± 4 aphids and 53 ± 4 aphids on NIL+ and NIL−, respectively.

Antixenosis affects the behavior and settling preference of aphids and was tested using a choice assay. We found that aphid settling was not significantly different between NILs

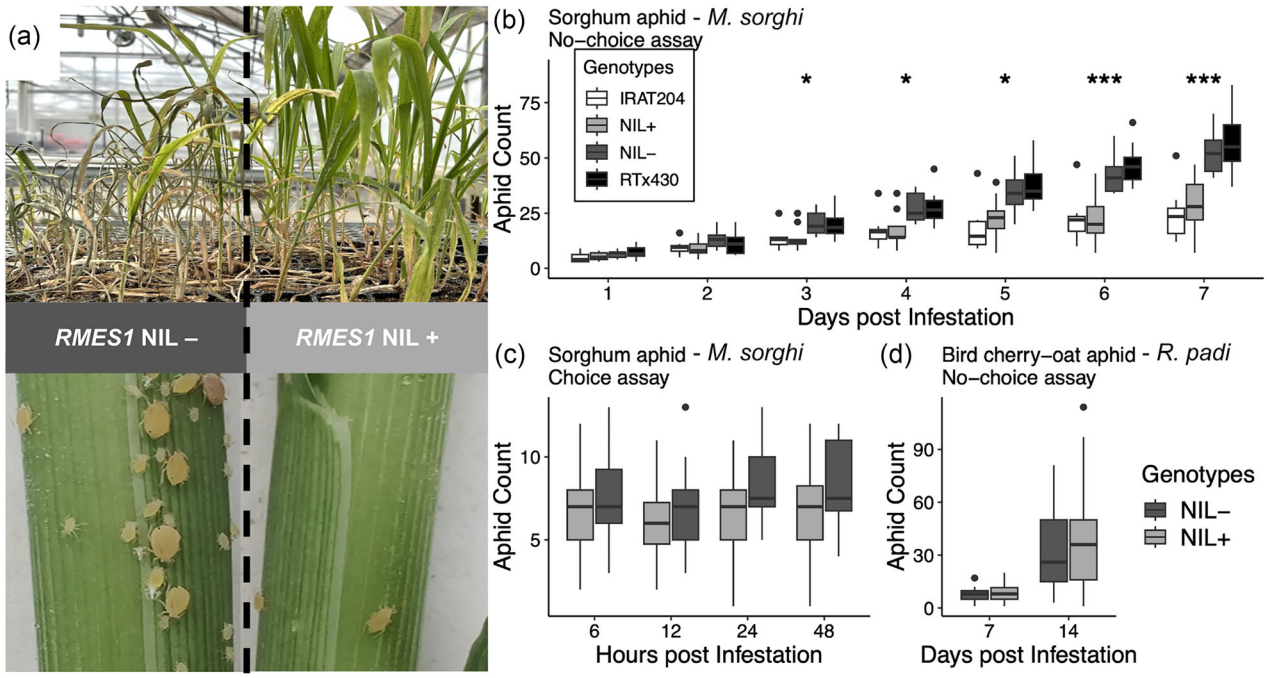

FIGURE 2 *Resistance to Melanaphis sorghi 1* (*RMES1*) provides antibiosis-resistance to sorghum aphid but not to other cereal aphids. (a) Representative photographs of sorghum aphid infestation on *RMES1* near-isogenic lines (NILs). (b) No-choice assay of sorghum aphid over 7-day infestation on NILs and parent genotypes ($N = 9$, $df = 15$). (c) Choice assay of sorghum aphid counts over 48 h infestation on NILs ($N = 16$, $df = 29$). (d) No-choice assay of bird cherry-oat aphid at 7 days post infestation (dpi) and 14 dpi ($N = 18$, $df = 30$). Significant pairwise comparison between NILs (Student's *t*-test at each timepoint) shown with asterisks (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).

at any time point in the first 48 h of infestation (Student's *t*-test, $p = 0.1$, $df = 29$; Figure 2c). There was an effect of time on aphid settling (repeated measures ANOVA, $p = 0.01$, $df = 45$) but not genotype ($p = 0.3$, $df = 15$) or the interaction ($p = 0.3$, $df = 25$). This indicates that sorghum aphid settling choice is not determined by *RMES1* and suggests a lack of constitutively expressed epidermal or volatile features that deter aphid settling. The substantial antibiosis phenotype agrees with the expected observation under the hypothesis that *RMES1* is placing selection pressure on aphid populations.

3.3 | *RMES1* does not provide resistance to bird cherry-oat aphid

The presence of *RMES1* in the commonly used breeding line RTx2783 with greenbug resistance led us to the hypothesis that the locus provides resistance to other cereal *Aphididae* species (Armstrong et al., 2015). A no-choice assay of bird cherry-oat aphid on *RMES1* NILs showed no effect on reproduction over 7- and 14-day infestation (Student's *t*-test, $p = 0.3$, $df = 30$; Figure 2d). Aphid reproduction was lower for bird cherry-oat aphid than sorghum aphid, with aphid populations growing from 3 to an average 8 ± 1 aphids after 1 week of infestation and an average 30 ± 9 aphids after 2 weeks, respectively. This indicates *RMES1* is at least partially discriminant in providing cereal aphid resistance, although it may still play a role in greenbug resistance.

3.4 | GWAS with resequencing confirms that *RMES1* resistance allele is rare in global landraces

It was previously shown that the *RMES1*-associated SNP with the highest fixation signature in a Haitian breeding program (S06_2995581v3) was rare in a global diversity panel (Muleta et al., 2022). Having used GBS data and a breeding population to identify this *RMES1* associated SNP, it remains possible that an SNP in higher linkage disequilibrium (LD) with *RMES1* exists and is at detectable frequency in association panels. We retested the rare *RMES1* hypothesis by combining recently published phenotypes for sorghum aphid resistance in global panels of landraces and improved lines (SAP and BAP) with whole-genome resequencing data and the *S. bicolor* BTx623v5.1 reference and performed genome-wide association analyses (GLM; Poosapati et al., 2022). We did not observe a peak at *RMES1* on Chr06 (Figure 3a; Supporting Information Data S1). We included the major association for resistance, S09_61521444, as a fixed-effect covariate in our GLM model and confirmed that the Chr06 locus was not

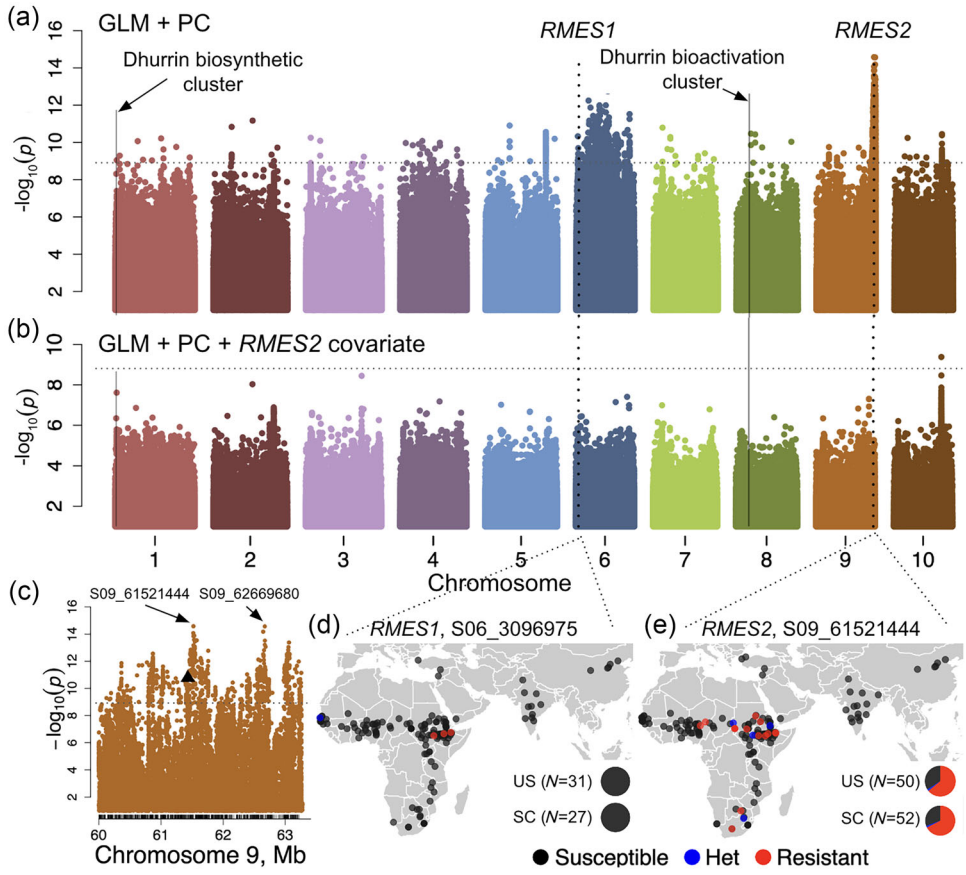

FIGURE 3 Genome-wide associations with sorghum aphid resistance in global diversity panels show *Resistance to Melanaphis sorghi 1* (*RMES1*) resistance is globally rare, while *Resistance to Melanaphis sorghi 2* (*RMES2*) is globally common. (a) Association of resequencing variants determined with a general linear model (GLM) that included principal components (PCs) 1–3 as fixed effects and phenotypes from Poosapati et al., 2022. (b) Association of resequencing variants determined with a GLM that included PC 1–3 of population structure and the peak *RMES2* single nucleotide polymorphism (SNP) from the GLM (S09_61521444) as fixed effect covariate. (c) Chr09, where Chr is chromosome, associations at *RMES2* highlighting the two most significant SNPs from the GLM + PC. The previously reported variant in the *SbWRKY86* gene model (S09_57630053v3) is indicated by the black triangle. Black lines below the associations indicate gene models in the region. Dotted grey lines (horizontal) indicate Bonferroni-corrected threshold. (d) Distribution of *RMES1*-associated SNP previously identified (Muleta et al., 2022). (e) Distribution of *RMES2*-associated SNP identified in the present study. Resequencing data available for historic breeding germplasm (US) and lines from the sorghum conversion program (SC) was used to estimate the frequency of alleles in foundational US germplasm.

significant after controlling for potentially confounding variation (Figure 3b; Supporting Information Data S2). An association at S01_1536310 was 358 kb from the cyanogenic biosynthetic gene cluster, between 1.07 and 1.18 Mb on Chr01 (Figure 3b) (Hayes et al., 2015). Additional loci on Chrs 2, 3, and 10 were apparent when the major Chr09 HPR was accounted for and are candidates for additional quantitative HPR sources (Table 1).

3.5 | *RMES2* resistance allele is common in global diversity lines

RMES2 was previously reported as a source of HPR in the combined association panel, and *SbWRKY86* was proposed as the causal gene due to the strongest associations falling within the gene model (Poosapati et al., 2022). In order to test the hypotheses on *RMES2* genomic position and inform marker development, we used resequencing data, which contains ~200 times more variants than previous GBS datasets, and generated new genome-wide associations to reanalyze Chr09. We found the peak association ($p < 10^{-14}$) at S09_61521444 (Figure 3a) for the resistant-associated reference allele (T) was present in 213 of 665 genotypes (32%). The second strongest association at *RMES2* was found 1.1 Mb from the peak association at S09_62669680 ($p < 10^{-14}$) (Figure 3c; Supporting Information Data S1). The previously reported peak association (S09_57630053v3 / S09_61433682v5) located in the 3′ untranslated region of *SbWRKY86* (Sobic.009G238200) was 89 kb from the peak association and remained significant ($p < 10^{-10}$) (Figure 3c) (Poosapati et al., 2022).

TABLE 1 Loci with significant associations with resistance (corresponding to Figures 3 and 4b,c).

Marker	Chr	Position (BTx623v5.1)	*p* value	Association analysis
S09_61521444	9	61521444	2.64E-15	SAP + BAP
S09_62669680	9	62669680	2.71E-15	SAP + BAP
S10_50970852	10	50970852	4.13E-10	SAP + BAP, *RMES2* fixed covariate
S03_59780623	3	59780623	3.58E-09	SAP + BAP, *RMES2* fixed covariate
S02_41893124	2	41893124	9.24E-09	SAP + BAP, *RMES2* fixed covariate
S01_1536310	1	1536310	2.43E-08	SAP + BAP, *RMES2* fixed covariate
S09_61988551	9	61988551	8.76E-13	Segregating F2, mid-season survival
S06_2170466	6	2170466	8.33E-12	Segregating F2, mid-season survival
S08_48830246	8	48830246	6.00E-13	Segregating F2, mid-season survival
S08_11769483	8	11769483	2.46E-14	Segregating F2, early-season survival
S01_78081631	1	78081631	5.00E-16	Segregating F2, early-season survival
S03_66114610	3	66114610	1.53E-13	Segregating F2, early-season survival
S09_59794306	9	59794306	5.54E-13	Segregating F2, early-season survival
S01_1253672	1	1253672	1.01E-12	Segregating F2, early-season survival

Abbreviations: BAP, Bioenergy Association Panel; Chr, chromosome; *RMES2*, *Resistance to Melanaphis sorghi2*; SAP, Sorghum Association Panel.

The *RMES1* allele identified via selection signatures in resistant breeding lines was previously shown to be globally rare and unique to East African landraces (Muleta et al., 2022). The presence of the resistant *RMES2* allele (S09_61521444) in 32% of association panel genotypes suggested that it is globally common relative to *RMES1*. We used resequencing data for genotypes with known landrace or breeding origin to test this hypothesis. Of 145 sorghum landraces, the resistant *RMES1* (S06_3096975) was present in just three (2.1%), while the *RMES2* allele was present in 13 (9.0%) lines (Figure 3d,e; Supporting Information Data S3). The geographic distribution of *RMES2* was broader than *RMES1* across Africa; however, the highest concentration was in East Africa. The resistant *RMES2* allele was present at a higher frequency in historic US breeding lines (67.3%) and sorghum conversion lines (34.0%) than in landraces, whereas the resistant *RMES1* allele was absent in both historic sets.

3.6 | Both *RMES1* and *RMES2* are being selected in a globally admixed breeding population

The prevalence of *RMES2* in global germplasm and its association with aphid resistance supports its availability and value for breeding programs. The fixation scan previously reported on the phenotypically resistant admixed breeding germplasm was reanalyzed in light of *RMES2* (Muleta et al., 2022). We found a strongly selected variant (S09_61842248, $p < 10^{-10}$) at *RMES2* and ~321 kb from the peak association panel variant S09_61521444 (Figure 4a). While *RMES2* variants were significant, the most significant variant on Chr09 was at S9_53496194 ($p < 10^{-14}$) and 8 Mb from *RMES2*.

TABLE 2 Loci with significant fixation signatures in globally admixed breeding population (corresponding to Figure 4a).

Marker	Chr	Pos (BTx623v5.1)
S6_3096975	6	3096975
S6_2993832	6	2993832
S2_60183555	2	60183555
S8_11803397	8	11803397
S9_53496194	9	53496194
S7_51523431	7	51523431
S9_61842248	9	61842248

Abbreviation: Chr, chromosome.

In addition to *RMES1* and *RMES2*, outliers of fixation signatures colocated with staygreen (*Stg3a*, S2_60183555) QTL and 1.6 Mb from the dhurrin bioactivation gene cluster (S08_11803397) (Table 2). These loci could represent additional traits under selection in the breeding program (e.g., post-flowering drought tolerance and forage quality). However, they may also play a role in sorghum aphid HPR.

3.7 | *RMES1* and *RMES2* provide resistance in segregating F_2 population founded on globally admixed genotypes

RMES1 was mapped in biparental populations for aphid resistance and through fixations signatures in the aphid resistant admixed germplasm, whereas *RMES2* has been mapped in association panels for aphid resistance (Muleta et al., 2022; Poosapati et al., 2022; F. Wang et al., 2013). We used a highly recombinant F_2 population derived from crosses between

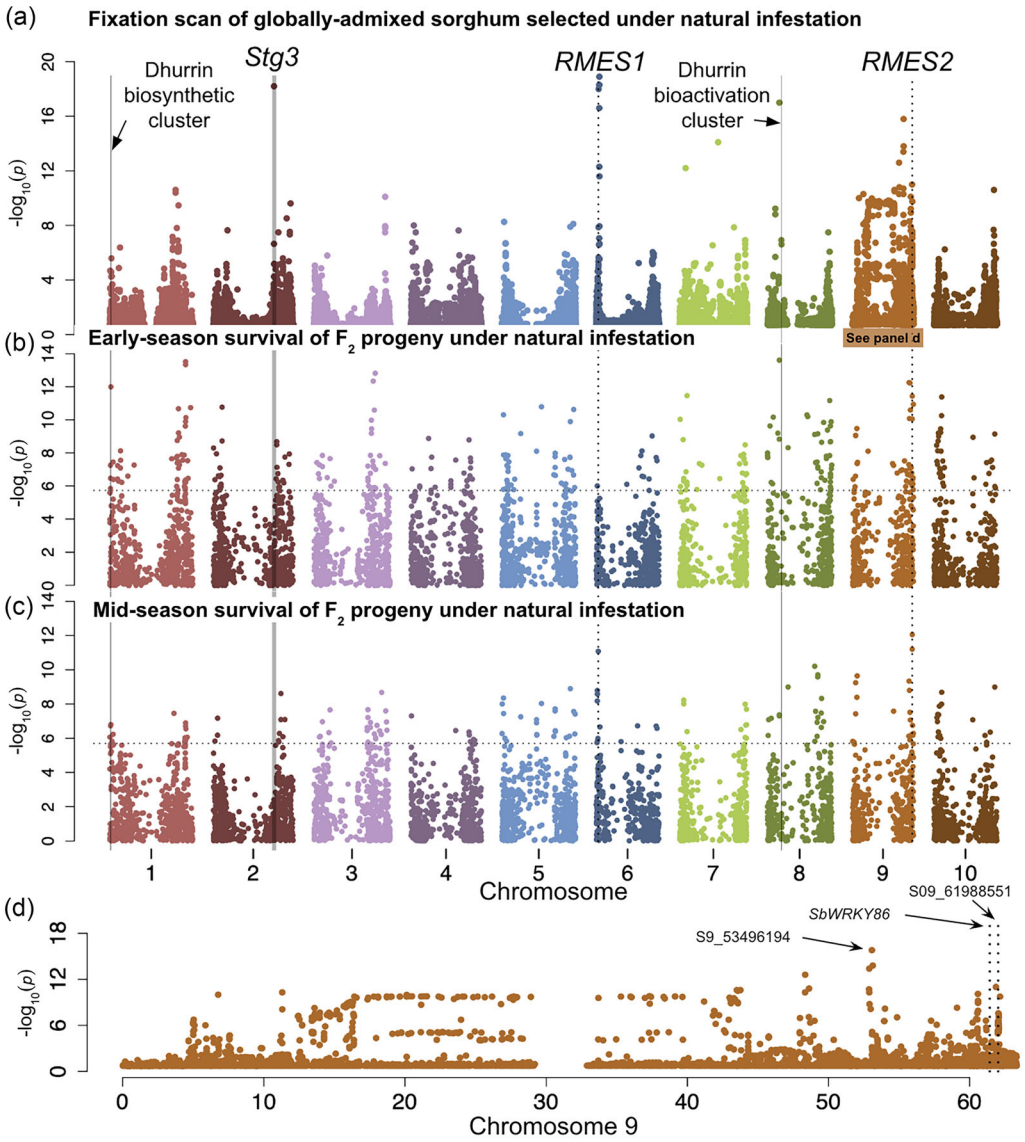

FIGURE 4 Both *Resistance to Melanaphis sorghi 1* (*RMES1*) and *Resistance to Melanaphis sorghi 2* (*RMES2*) contribute to aphid resistance in a globally admixed breeding population. (a) Fixation scores (F_{ST}) determined for the phenotypically resistant admixed germplasm compared with the global diversity panel. Data replotted from Muleta et al. (2022). Notable signatures are indicated with shaded lines (dhurrin biosynthesis and activation, *Stg3a* QTL region) (Cicek & Esen, 1998; Sanchez et al., 2002) or dashed lines (*RMES1* and *RMES2*). (b and c) Associations with resistance under heavy aphid infestation in an F_2 population of admixed germplasm segregating for resistance. (b) Survival at flowering initiation associations. (c) Survival at booting associations. The two strongest associations at booting for *RMES1* and *RMES2* are indicated by black dotted lines for all panels. Dotted gray lines (horizontal) indicate the Bonferroni-corrected threshold. (d) F_{ST} scores on Chr09, where Chr is chromosome. S06_3096975 and S09_61988551 are used to indicate *RMES1* and *RMES2* in panels a–c.

resistant admixed genotypes and African landraces that were phenotyped for survival under heavy natural infestation of sorghum aphid in order to test hypotheses on global genetic architecture contributing to aphid resistance at early-season (flowering initiation) and mid-season (booting).

Despite expecting *RMES1* to contribute resistance throughout all growth stages due to antibiosis observed at the seedling stage, our GLM analysis showed that only *RMES2* (S09_59794306, $p < 10^{-12}$) was associated with resistance early in development (Figure 4b; Supporting Information Data S4). An association on Chr01 (S01_1253672, $p < 10^{-11}$) was 76 kb from the dhurrin biosynthetic cluster, while an association on Chr08 (S08_11769483, $p < 10^{-13}$) was 1.6 Mb from the dhurrin bioactivation cluster. Other early-season resistance associations on Chr01 (S01_78081631, $p < 10^{-13}$) and Chr03 (S03_66114610, $p < 10^{-12}$) indicate that HPR is polygenic at early growth stages. In contrast to early-season, the two strongest mid-season associations with resistance were found at *RMES2* (S09_61988551, $p < 10^{-11}$) and *RMES1* (S06_2170466, $p < 10^{-11}$) (Figure 4c; Supporting

Information Data S5). There was no significant interaction between the two loci, indicating no epistasis detectable in this population. The third-most significant association at booting was on Chr08 at S08_48830246 ($p < 10^{-10}$). Notably, *RMES2* was the only QTL associated with resistance at both growth stages, and there was no colocalization of dhurrin-related QTL with resistance associations at the later growth stage.

4 | DISCUSSION

Here, we characterized the major source of aphid HPR in a sorghum breeders toolbox (*RMES1*) and showed evidence that additional sources, *RMES2* and dhurrin biosynthesis QTL, complement the genetic architecture underlying global sorghum aphid resistance. We found that *RMES1* HPR provides antibiosis-resistance to sorghum aphid but not bird cherry-oat aphid. Next, we determined a strong likelihood that *RMES2* is present in breeding programs. Finally, we describe the collective contribution of *RMES1* and *RMES2* in a Haitian breeding program where breeding for sorghum aphid resistance is a priority. Co-localizations of aphid resistance associations with dhurrin pathway genes suggest cyanogenic potential as a quantitative source of resistance.

4.1 | Isolating *RMES1* in NILs allowed the phenotype and threat of biotype shift to be defined

Breeding-focused research on HPR often identifies genetically dissimilar germplasm differing for traits such as antibiosis or tolerance, which prevents mechanisms from being resolved at the genetic (e.g., QTL) level. It is important to mendelize traits using NILs, induced mutation, or gene editing in order to test gene-specific hypotheses that untangle the many levels of HPR and elucidate individual mechanisms. The majority isogenic background and single resistance locus segregating in our NILs allowed us to define *RMES1* antibiosis resistance for sorghum aphid (Muleta et al., 2022). Future investigations of the molecular mechanism will benefit from continued population development of these NILs.

The common breeding line RTx2783 contains the resistant *RMES1* allele and showed tolerance and resistance to sorghum aphid and greenbug, leading to our hypothesis that *RMES1* is pleiotropic for these traits (Armstrong et al., 2015). While bird cherry-oat aphid is not an agriculturally significant pest on sorghum, it can infest sorghum and allowed us to test the hypothesis that *RMES1* provides resistance to other cereal aphids (Michaud et al., 2017). NILs had no effect on bird cherry-oat aphid reproduction in the no-choice assay (Figure 2d) showing that the molecular mechanism of resistance is not shared across all sorghum–*Aphididae* systems. Resistance to greenbug has not been associated with the *RMES1* region in mapping studies to our knowledge, supporting RTx2783 greenbug resistance being derived from non-*RMES1* sources (https://aussorgm.org.au/sorghum-qtl-atlas/; Harris-Shultz et al., 2020). *RMES1* is the only source of sorghum aphid HPR with marker technology and known to be used in public and private sorghum breeding programs (Muleta et al., 2022). Biotypic variation for *RMES1* virulence has not been reported. However, *RMES1* selection pressure on sorghum aphid, wide deployment in cultivars, and history of biotype shifts in other cereal-aphid systems lead us to conclude that *RMES1* is vulnerable to a breakdown of resistance.

The molecular mechanism of *RMES1* remains to be tested, but two hypotheses have been put forward. One candidate is *SbCAS1* (Sobic.006G016900), a β-cyanoalanine synthase gene involved in the detoxification of hydrogen cyanide (HCN) produced by the cyanogenic glucoside, dhurrin (Gleadow et al., 2021). Cyanogenesis is involved in antibiosis-resistance to the Fall armyworm (*Spodoptera frugiperda*) in sorghum; however, involvement in aphid resistance has not been tested (Gruss et al., 2022). A change in HCN detoxification strategy could have autotoxic consequences for the host, and the capacity to detoxify HCN is unlikely to vary significantly among cereal aphids, that is, bird cherry-oat aphid and sorghum aphid (Poulton, 1990). The other set of candidates involves a cluster of NLR receptors, which recognize molecular patterns of infestation and activate host defenses, and are predicted in the region of *RMES1* in the BTx623 (susceptible) and RTx2783 (resistant) genomes (Muleta et al., 2022; Snoeck et al., 2022; B. Wang et al., 2021). These are strong candidates for causal genes due to numerous reports of NLR genes driving resistance in aphids and other pest systems (Boissot, 2023; Dogimont et al., 2014; Snoeck et al., 2022). This class of resistance mechanisms is expected to be less durable if the selection pressure is high and the herbivore-associated molecular pattern can withstand mutations to evade the host receptor. Such "gene-for-gene" dynamics agree with the *Aphididae*-discriminant phenotype with sorghum aphid and bird cherry-oat aphid and could lead to boom-and-bust cycles similar to those seen in cereal-rust systems (Dogimont et al., 2010; Flor, 1971; Mundt, 2018; Thompson & Burdon, 1992). One or both cyanogenesis and NLR mechanisms could underlie *RMES1*.

As future studies establish the relative durability of antibiosis, antixenosis, and tolerance mechanisms, knowledge of *RMES1*-antibiosis will inform how to combine and utilize all HPR available. RTx2783 appears to harbor additional tolerance and HPR sources, as it was reported to retain growth despite moderate aphid infestation (Limaje et al., 2018). Alternatively, pleiotropic interactions from *RMES1* may contribute additional tolerance mechanisms in different

backgrounds. Regardless of the pleiotropic tolerance hypothesis, a significant reduction in the fecundity of aphids on *RMES1* NILs demonstrates a selection pressure on sorghum aphid and increases the likelihood of a shift toward virulent biotypes. This HPR source should not be solely relied upon for crop protection as there is precedence for monogenic HPR breakdown in cereal-aphid systems (Harris-Shultz et al., 2020).

4.2 | *RMES2* is moderately common in global landraces and contributes HPR in a diversity panel

Previously published high-quality resistance phenotyping of two association panels in conjunction with dense genotyping provided the opportunity to test hypotheses on architecture-contributing resistance while also informing *RMES2* marker development, which will provide a second breeder-friendly tool. The two strongest associations with GBS SNPs were inside the gene model of *SbWRKY86*, a transcription factor responsive to aphid infestation (Kiani & Szczepaniec, 2018; Poosapati et al., 2022). We found two regions, 87.8 kb and 1.24 Mb from *SbWRKY86*, which had a higher association than the original SNP in our analysis (Figure 3a). One methodological hypothesis for discrepancies is differences between the previous panel of 697 genotypes and our subset of 665 genotypes with available resequencing. Another hypothesis is that genotypic variation at *SbWRKY86* is not causal for *RMES2* HPR, but *trans*-regulating elements modulate this transcription factor (Atamian et al., 2012; P. Li et al., 2015). Finally, it is possible that *SbWRKY86* is not involved in *RMES2* resistance and one or both of the QTL (S09_61521444, S09_62669680) are in higher LD with the causal gene(s). This QTL is in a gene-dense region of Chr09 with 278 genes annotated between 61 and 63 Mb, demonstrating the need for fine-mapping or variant testing to confirm or exclude the hypothesis that *SbWRKY86* underlies *RMES2* resistance.

The peak *RMES2* variant was at high frequency in global germplasm as well as dispersed across Africa (Figure 3e). The resistant allele's high frequency in historic breeding germplasm and sorghum conversion lines indicates that *RMES2* has been present in North America for over half a century and is widely available for breeding in diverse and adapted backgrounds (Stephens et al., 1967). The *RMES2* QTL is within 1 Mb of the dwarfing gene *Dw1*, whose non-functional allele was selected for in the development of temperate US varieties (Hilley et al., 2016). One explanation for the high frequency of the resistant *RMES2* allele in early US breeding lines is that it was present in the original *dw1* mutant line Dwarf Yellow Milo, which was widely used in breeding programs for reduced lodging and mechanized harvesting (Karper & Quinby, 1946). This is promising for breeders seeking additional HPR for cultivar development and will accelerate deployment when markers become available. *RMES2* KASP marker development is currently underway.

4.3 | *RMES2* bolsters *RMES1* to improve global HPR durability

The Chibas breeding program at the University of Quisqueya in Haiti breeds mixed-use temperate sorghums in high aphid-pressure environments (Muleta et al., 2022). A breeding scheme that maximizes recombination with globally diverse founders of the breeding population provided strong statistical power to identify novel resistance associations that may be undetectable in association panels (e.g., *RMES1*) (Cockram & Mackay, 2018). *RMES1* and *RMES2* were the most significant QTLs for mid-season survival under infestation. However, *RMES1* was not associated with early-season survival under infestation (Figure 4b,c). One hypothesis is that there were abiotic (nutrient and environmental) or biotic factors other than sorghum aphid pressure determining plant fitness at early stages different from late developmental stages. Another hypothesis is that the *RMES1* phenotype is developmentally regulated akin to adult plant resistance. However, antibiosis was seen in the seedling stage in controlled greenhouse experiments (Figure 2a,b), and *RMES1* was first mapped in a biparental population at the seedling stage in controlled settings (F. Wang et al., 2013). It is also possible that *RMES1* resistance is present throughout development, where its relative HPR contribution increases with maturity but is undetectable in early-season field environments.

A common HPR source for early and late resistance would be expected to have positive selection and would indicate collective benefit to resistance. Agreeing with this hypothesis, variants at *RMES2* show selection signatures (Figure 4a). The use of multiple resistance genes in a single genotype, or "gene pyramids," reduces the likelihood of pest evolution overcoming the cumulative resistance and bolsters the overall durability of HPR (Fuchs, 2017; Mundt, 2018; Palloix et al., 2009; Quenouille et al., 2013). The selection of *RMES2* with *RMES1*, their additive contribution to resistance in the Haitian breeding program, and the presence of *RMES2* in global and historic sorghum genotypes led us to conclude that *RMES2* is bolstering *RMES1* HPR durability globally in breeding programs where sorghum aphid resistance is a target trait.

4.4 | Dhurrin and other candidate sources of quantitative and seedling resistance

We found evidence of additional sources of resistance in the association panels when controlling for the major *RMES2*

association and in globally admixed F_2 lines, particularly at the seedling stage, that may be sources of quantitative or early-season HPR. The cyanogenic glucoside dhurrin is known to provide resistance to chewing insects via disruption of respiration and may be an aphid resistance mechanism, although not proven (Dreyer et al., 1981; Gruss et al., 2022). As a major target trait in forage breeding, variation for dhurrin biosynthesis and bioactivation have been well studied, with major variation mapped to Chr01 and Chr08, respectively (Hayes et al., 2015). The biosynthetic gene cluster (*CYPE71E1*, *CYPE79A1*, and *UGT85B1*) coincided with associations on Chr01 for resistance confounded by *RMES2* (Figure 3b) and early-season resistance (Figure 4b). Dhurrin content is higher in seedlings, and biosynthesis genes are highly expressed through vegetative development, which may explain the lack of association for resistance at maturity (Figure 4c) (Gleadow et al., 2021; Halkier & Møller, 1989). An association with early-season resistance on Chr08 is selected for; however, it falls over 1 Mb away from the bioactivation gene cluster and may represent a non-cyanogenic mechanism (Figure 4a,b; Tables 1 and 2). Further investigations are warranted of dhurrin anabolism and catabolism as a mechanism of resistance against sorghum aphid.

Epicuticular waxes are known to affect aphid infestation as well as other biotic and abiotic traits in sorghum (Premachandra et al., 1994; Weibel & Starks, 1986). Mutant genotypes lacking waxy depositions, such as *bloomless2*, which alters monoacyl glycerol and 32-C-alcohol wax profiles, are preferred for sorghum aphid feeding compared to wild-type sorghum but do not affect aphid reproduction (Cardona et al., 2022). Despite the established relationship between bloomless mutants and aphid HPR, natural variation for waxes contributing to HPR has not been reported. Natural variation for epicuticular wax in an association panel was identified on Chr03 (~66.6 Mb) and within 0.5 Mb of the S03_66114610 association for early-season resistance (Figure 4b; Table 1) (Elango et al., 2020). Wax deposition in sorghum is believed to be pleiotropic, benefitting water use efficiency traits, reducing forage digestibility, and preventing foliar pathogens (Burow et al., 2008; Cummins & Sudweeks, 1976; Jenks et al., 1994; Premachandra et al., 1994). Additional pleiotropy for sorghum aphid resistance would need to be considered in using this as a source of HPR.

4.5 | Next steps to mechanisms and global durability

Here, we elucidated antibiosis-resistance provided by *RMES1*, which places selection pressure on sorghum aphid populations. However, additional sources of resistance, including *RMES2*, are increasing the global durability of HPR. A molecular marker for *RMES2* will allow breeders to select for gene pyramids more easily during cultivar development. Genomic and functional characterization of both *RMES1* and *RMES2*, as well as validation of dhurrin as an HPR source, will improve the molecular understanding of the genotype to phenotype map and inform breeding strategies. Global agricultural resilience will benefit from sustainable sorghum production that is durably aphid resistant as climate change and anthropogenic factors continue to challenge food security around the world.

AUTHOR CONTRIBUTIONS

Carl VanGessel: Conceptualization; data curation; formal analysis; methodology; validation; visualization; writing—original draft; writing—review and editing. **Brian Rice**: Conceptualization; formal analysis; methodology; writing—review and editing. **Jean Rigaud Charles**: Resources. **Terry J. Felderhoff**: Resources; writing—review and editing. **Gael Pressoir**: Conceptualization; funding acquisition; methodology; project administration; resources; writing—review and editing. **Vamsi Nalam**: Conceptualization; methodology; project administration; resources; writing—review and editing. **Geoffrey Morris**: Conceptualization; funding acquisition; methodology; project administration; resources; supervision; writing—original draft; writing—review and editing.

ACKNOWLEDGMENTS

This study is made possible by the support of the American People provided to the Feed the Future Innovation Lab for Collaborative Research on Sorghum and Millet through the United States Agency for International Development (USAID) under Cooperative Agreement No. AID-OAA-A-13-00047. The contents are the sole responsibility of the authors and do not necessarily reflect the views of USAID or the United States Government. Phenotyping and evaluation also received additional support from the Haitian Ministry of Agriculture, Natural Resources, and Rural Development through PITAG project (supported by the Interamerican development bank). We thank three anonymous reviewers for suggestions that improved the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Raw whole-genome sequencing reads, association panel genotype data, Haitian genotype data, and geographic distribution data have been deposited at Dryad Digital Repository (https://doi.org/10.5061/dryad.rv15dv4f6).

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SUPPORTING INFORMATION

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How to cite this article: VanGessel, C., Rice, B., Felderhoff, T. J., Charles, J. R., Pressoir, G., Nalam, V., & Morris, G. P. (2024). Globally deployed sorghum aphid resistance gene *RMES1* is vulnerable to biotype shifts but is bolstered by *RMES2*. *The Plant Genome*, e20452. https://doi.org/10.1002/tpg2.20452