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Utilizing genetic variation in perennial sorghum to improve host plant resistance to aphids

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With growing concerns over the sustainability of conventional farming systems, perennial crops offer an environmentally friendly and resilient alternative for long-term agricultural production. Perennial grain crops provide numerous benefits, such as low input investment, reduced tillage, soil conservation, better carbon sequestration, sustainable yields, and enhanced biodiversity support. Sorghum (*Sorghum bicolor*) is the fifth most-grown cereal crop grown for food, fuel, and food grain in the world. The development of perennial sorghum offers a substitute for traditional annual sorghum crops by providing long-term environmental, economic, and agronomic benefits. Sugarcane aphid (SCA; *Melanaphis sacchari*), a phloem-feeder, is considered a major threat to sorghum production. Since its first report in 2013, it caused $40.95 million in losses in South Texas alone by 2015, accounting for about 19% of the total value of sorghum production in the region. In this study, we screened diverse perennial sorghum genotypes using no-choice and choice assays to determine their innate antibiosis and antixenosis resistance levels to SCAs. Based on aphid reproduction and plant damage rating, no-choice bioassay classified the 43 perennial sorghum genotypes into four clusters: highly susceptible, moderately susceptible, moderately resistant, and highly resistant. To further investigate the resistance mechanisms, we selected two genotypes, X999 > R485 (SCA-resistant) and PR376 ~ Tift241 (SCA-susceptible) that showed the greatest variation in resistance to SCA, for subsequent experiments. Choice bioassay results indicated that aphids chose PR376 ~ Tift241 for settlement, whereas no significant preference was observed for X999 > R485 compared to the control genotype. Electrical penetration graph (EPG) results demonstrated that aphids feeding on the SCA-resistant genotype spent significantly less time in the phloem phase than the susceptible genotype and control plants. The identification of SCA-resistant perennial sorghum genotypes will be valuable for future sorghum breeding programs in managing this economically important pest.

Keywords Antibiosis, Antixenosis, EPG, Phloem, Sorghum, Sugarcane aphids

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the major cereals grown worldwide, with 57 million tons of production in 2023. The United States of America (USA) is the largest producer of sorghum, followed by Nigeria and India. Sorghum is grown in different agroecological zones of the world and displays tolerance to drought and water stress[1]. Due to its versatile nature, it is processed as a major food grain in arid and semi-arid regions of the developing world. However, this carbohydrate-rich crop is mainly used for bioethanol and livestock feed production in the western regions of the world[2]. Numerous health benefits of sorghum have evoked its rising demand at the global level[3–5].

Like other crops, sorghum bears many biotic and abiotic stresses in its natural environment. Sugarcane aphid (SCA; *Melanaphis sacchari* (Zehntner)) is a serious pest of sorghum in North America, which has significantly impacted its production since the first outbreak reported in Texas and Louisiana in 2013[6–9]. In South Texas, the SCA outbreak led to total economic losses of $40.95 million from 2013–2015[10]. Currently, SCA is reported in more than 20 sorghum-producing states of the USA (Eddmaps, 2023). Like other aphids, SCA has specialized piercing-sucking structures known as stylets. On piercing the plant tissue, SCA consumes the phloem sap, depleting plant nutrients. In addition, the deposition of honeydew by aphids on the leaf facilitates the development of sooty mold, affecting the photosynthetic ability of the plant and incurring yield losses of 50% to nearly 100% under heavy infestations[11,12].

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With climate oscillations, present annual cultivars are becoming increasingly susceptible to stress[13]. This presents an opportunity for utilizing wild germplasm and perennial crops for the increasing demands in agriculture[14,15]. Perennial crops provide numerous environmental, economic, and agronomic advantages. Their deep root systems help retain water and increase water efficiency, decrease soil erosion, maintain land stability, and reduce nutrient runoff. Since there is no need for annual replantation, perennial crops also offer lower economic inputs and reduces labor costs[16–19]. Currently, *S. bicolor* is the most exploited annual germplasm for sorghum cultivars. The two perennial species classified in the same genus are *S. halepense and S. propinqumm*. *S. halepense* is widespread across the Eastern Hemisphere and is considered as an invasive species and a noxious weed in 16 and 20 states of the USA, respectively[20–22]. In contrast, *S. propinqumm* is restricted to Southeast Asia and thrives in tropical environments. *S. halepense* (2n = 40) is a weedy perennial species owing to its well-developed creeping rhizome formation. Aside from its weediness, it is used as an important fodder in many subtropical areas. However, during periods of vigorous growth, drought, or following frost, it can accumulate high levels of cyanogenic compounds, making it potentially toxic to livestock[23]. *S. halepense* has been widely used in sorghum breeding programs to cultivate the desirable traits of perennial sorghum into annual sorghum. The use of wild germplasm to develop perennial cultivars that will be resistant to SCA adds another facet to the benefits of perennial grains. The economic and environmental implications of this strategy include reduced investment in pesticides and lower residues and carbon footprint of agriculture systems[24,25].

As recent research has made great strides toward developing perennial versions of major grain crops, it provides a great opportunity to utilize host plant resistance (HPR) in the control strategies of developing cultivars[26]. HPR is the practice of intentionally using resistant crop cultivars and avoiding the use of varieties with high levels of susceptibility to an arthropod pest to reduce the negative impacts of pests or diseases on crop production[27]. First recognized as a pest control strategy in the early twentieth century, HPR has evolved into a vital component of integrated pest management systems[28]. Focused identification of germplasm strategy (FIGS) provides a way to maximize the likelihood of identifying plant genotypes with adaptive traits from wild populations[29]. Identifying and characterizing the mechanisms of pest resistance is important to understand the plant–insect interactions. This also provides us with the opportunity for effective deployment. Plant resistance towards pests can be categorized into three categories: antixenosis, antibiosis, and tolerance. Plants displaying antixenosis (non-preference) deter insects by affecting their host plant choice for feeding, oviposition, or colonization using various chemical, physical, and morphological factors. Antibiosis influences insect's life biology traits, negatively impacting their development, fecundity, survival, and fitness on host plants. In contrast, tolerance is a plant characteristic that enables it to sustain insect populations while withholding significant insect damage on growth, development, and yield[30–33]. These mechanisms often work synergistically, providing plants with multifaceted resistance to pest pressures. Ongaratto et al. (2021)[34] found soybean genotypes that exhibited antibiosis and antixenosis resistance against *Anticarsia gemmatalis* and compared the life-history traits among the highly resistant genotypes, demonstrating how multiple mechanisms can collectively enhance plant defenses. Genetic variation in crops has been used to explore resistant factors and incorporate them into modern cultivars. Several accessions of *Brassica carinata* were screened against *Myzus persicae* infestation to discover resistant and susceptible sources for interspecific hybridization in *Brassica* crops[35]. A similar strategy was used in barley (*Hordeum vulgare* L.), where genetic diversity and population structure of wild barley genotypes were assessed using microsatellite markers against *Rhopalosiphum maidis* to provide foundational work for corn leaf aphid resistance breeding schemes[36].

Aphids recognize hosts by inserting their stylets into the plant tissue and secreting saliva into the plants. The stylet penetration in a plant is a pivotal parameter in assessing host acceptance by aphids. The probing behavior of aphids can be monitored using the Electrical Penetration Graph (EPG) technique, which allows tracking stylet movement in different plant tissues in real-time by characterized waveforms of fluctuating voltage[37]. The four major characteristic waveforms include the pathway phase, non-probing phase, phloem phase, and xylem phase. The pathway phase constitutes the time spent navigating through the apoplast to target the sieve element phase. In the no-probing phase, aphids stop actively penetrating the plant tissue and assess the cues for host suitability[38,39]. As the name suggests, aphids feed in the xylem and phloem tissues during the xylem and phloem phases, respectively. Numerous studies have used this electrophysiological technique to understand the host-plant interaction. Previously, we have shown that SCA feeding on the resistant sorghum lines spent significantly less time in the phloem phase and more time in the pathway phase[40,41]. Similarly, MacWilliams et al. (2023)[42] discussed that cowpea aphids behaved in the same pattern on the resistant cowpea line, indicating that the plant's resistance diminishes the aphid's ability to obtain nutrients, affecting its survival and reproductive success.

Finding sources of resistance in perennial sorghum is useful from an ecological and financial standpoint, especially considering the destruction inflicted by SCA. Current agricultural systems will become more environmentally sustainable with the adoption of resistant SCA perennial sorghum cultivars. However, there is a lack of research findings related to SCA-resistant perennial sorghum genotypes. In this study, we screened various perennial sorghum genotypes against SCA infestation, with the aim to assess genetic variation in resistance in these genotypes (Table 1). We hypothesized that aphid bioassays will identify distinct sources of SCA resistance based on host plant resistance categories. The perennial sorghum genotypes were developed from the cross of *S. bicolor* and *S. bicolor* X *S. halepense* lines[43]. These genotypes were selected based on their biomass production. We employed no-choice and choice bioassays to determine the antibiosis and antixenosis resistance levels in these sorghum genotypes. To better understand the resistance mechanisms, we also monitored the feeding behavior of SCA on the highly resistant and highly susceptible perennial sorghum genotypes using the EPG technique.

Genotype	Perennial sorghum accession	Aphid count	Damage rating
G1	S1383 > 046C	46.50	3.25
G2	S3174 ~ B5 ~ R335A ~ PR225B ~ Tift81	49.90	3.50
G3	S1465 > R120D	37.17	3.33
G4	S1477 > R175A	30.67	3.75
G5	S3326 ~ C13 ~ R208A ~ PR259A ~ Tift126	24.33	3.50
G6	S3328 ~ C15 ~ R208A ~ PR259A	79.25	3.33
G8	S3200 ~ B1 ~ R379 ~ PR355C ~ Tift184	57.18	3.54
G9	S3326 > ….PR230F ~ Tift186	42.20	3.70
G10	S3174 ~ B5 ~ R335A ~ PR225B ~ Tift187	44.08	3.33
G11	S3323 ~ C1 ~ R207B ~ PR113A ~ Tift194	77.78	3.22
G12	S3326 > ….PR313C ~ Tift197 ~ Tift197	69.00	3.25
G13	S3331 ~ C13 ~ R214Z ~ PR283D ~ Tift202	53.09	4.09
G14	S3323 ~ C11 ~ R207B ~ PR287B ~ Tift216	55.63	3.82
G15	S2163 > R186B-R129G	46.54	4.63
G16	S3174 ~ A2 ~ R093C ~ PR284A ~ Tift221	84.50	3.42
G17	S3197 ~ A5 ~ R223 ~ PR231A ~ Tift225	42.67	3.42
G18	S1662 > R554B	33.75	3.25
G19	S1776 > R174	65.08	2.53
G20	S3181 ~ B1 ~ R100H ~ PR64A ~ Tift235	160.92	2.57
G21	S3190 ~ B6 ~ R363 ~ PR84 ~ Tift239	131.17	4.81
G22	S3323 ~ C11 ~ R207A ~ PR375 ~ Tift240	140.08	4.28
G23	S3323 ~ C11 ~ R207B ~ PR376 ~ Tift241	238.08	3.38
G25	S2097-4–137	123.78	3.22
G26	S3182 ~ B3 ~ R344 ~ PR217A ~ Tift253	73.92	2.93
G27	S3182 ~ B3 ~ R344 ~ PR217C ~ Tift257	108.67	3.26
G28	S3331 ~ A5 ~ R014B ~ PR150A ~ Tift258	81.00	2.92
G29	S3181 ~ B1 ~ R110K ~ PR66B ~ Tift259	85.50	3.17
G30	S3011-A1D1-PR28 ~ Tift275	99.83	3.07
G31	S3323 ~ C2 ~ R207Z ~ PR114A ~ Tift277	152.63	4.09
G32	S3188 ~ C8 ~ 119A ~ PR79 ~ Tift284	145.08	3.81
G33	TexasHCN_S1776 > R65	127.92	4.08
G34	S3326 ~ C13 ~ R208A ~ PR259A ~ Tift288	125.50	3.71
G35	S3323 ~ C11 ~ R207A ~ PR270 ~ Tift289	40.20	3.40
G36	S14PR > R181	28.56	3.11
G37	S3301 ~ B3 ~ R437B ~ PR104 ~ Tift296	121.67	2.71
G38	S3326 > ….PR230E ~ Tift310	43.78	3.11
G39	S3326 > ….PR230E ~ Tift311	28.80	3.00
G40	S3326 > ….PR313C ~ Tift322	86.917	2.69
G41	S3188 ~ A5 ~ R112 ~ PR339A ~ Tift338	44.33	3.00
G42	S3188 ~ C8 ~ 119A ~ PR79 ~ Tift26	121.83	1.91
G43	S1852 > 015E	32.70	4.00
G47	X999 > R305	84.75	4.16
G48	X999 > R485	26.10	3.60

Table 1. No-choice bioassay data accounting for the aphid numbers and damage incurred[71] on different perennial sorghum genotypes after SCA infestation.

Results

No-choice bioassay

Based on aphid counts, four distinct clusters were formed in the no-choice assay: highly susceptible, moderately susceptible, moderately resistant, and highly resistant genotypes (Fig. 1). The highly resistant cluster contained 12 genotypes (S1465 > R120D, S1477 > R175A, PR259A ~ Tift126, S1662 > R554B, S1776 > R174, PR270 ~ Tift289, PR230E ~ Tift310, PR230E ~ Tift311, PR339A ~ Tift338, S14PR > R181, S1852 > 015E, X999 > R485) while the highly susceptible cluster contained only one genotype (PR376 ~ Tift241). Integrating phenotypic data, a hierarchical cluster was formed based on aphid count and plant damage (Fig. 2). PR376 ~ Tift241 formed a separate cluster of highly susceptible genotypes. Another cluster of susceptible genotypes was observed with SC1345, a highly susceptible annual sorghum genotype, highlighting the susceptible perennial sorghum[44]. Regarding the resistant genotypes, a cluster of 8 genotypes was formed, which is distinct from SC265, implying

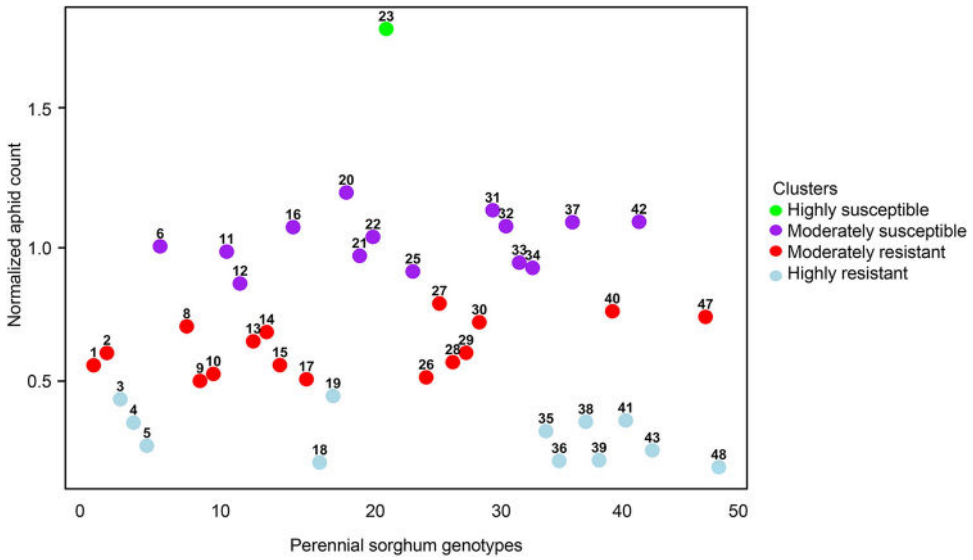

Fig. 1. *k*-means clustering analysis of the perennial sorghum genotypes based on aphid counts normalized with BTx623 (n = 9–12 per genotype). Four groups were designated as: Highly Susceptible (green), Moderately Susceptible (purple), Moderately Resistant (red), and Highly Resistant (blue) genotypes.

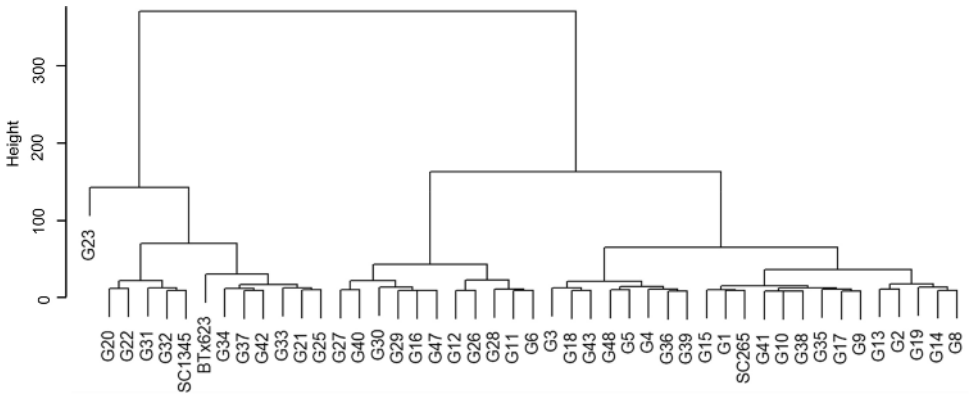

Fig. 2. Hierarchical clustering of the perennial sorghum genotypes based on aphid count and damage rating on SCA infestation on Euclidean mean distance using Ward's method (n = 9–12 per genotype).

the resistance level of this cluster to be higher than the annual resistant genotype[44,45]. Based on both genotypic and phenotypic traits, we identified five susceptible (PR376 ~ Tift241, PR64A ~ Tift235, PR114A ~ Tift277, PR79 ~ Tift284, PR375 ~ Tift240) and eight resistant genotypes (S1465 > R120D, S1477 > R175A, PR259A ~ Tift126, S1662 > R554B, S14PR > R181, PR230E ~ Tift311, S1852 > 015E, X999 > R485). The two perennial sorghum genotypes that showed greatest variation in resistance to SCA, PR376 ~ Tift241 (SCA-susceptible) and X999 < R485 (SCA-resistant), were used for the subsequent experiments and are denoted as Genotype23 (G23) and G48, respectively.

Choice bioassay

Results of the choice bioassay between lines of varying resistance showed that the number of aphids that choose to settle on G23 was significantly higher compared to the aphids that settled on BTx623, after the aphid release at both 6 h (df = 1; $P = 0.0169$) and 24 h (df = 1; $P = 0.0002$) (Fig. 3A). In contrast, the choice bioassay between G48 and BTx623 showed that aphids avoided selecting G48 for colonization and settlement and preferred to reside on BTx623 at both 6 h (df = 1; $P = 0.0010$) and 24 h (df = 1; $P < 0.0001$) after aphid release (Fig. 3B).

Feeding behavior parameters

Representative EPG waveform recordings were categorized into four different phases: pathway phase, phloem phase, xylem phase, and non-probing phase (Fig. 4). The EPG data demonstrated that aphid feeding varied in the pathway, phloem, and non-probing phases. The aphids spent significantly more time in the pathway phase in G48 compared with G23 and BTx623 genotypes (df = 2; $P = 0.0401$; Fig. 5A). There was no significant

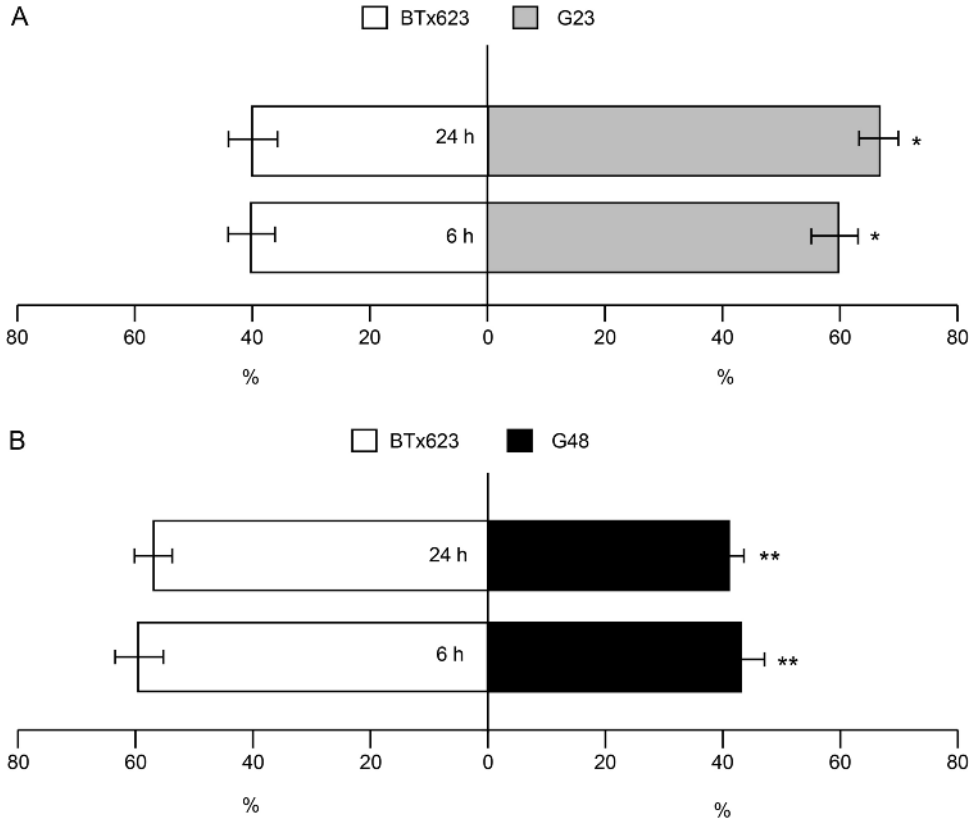

Fig. 3. Choice assay comparison of aphid preference for (**A**) BTx623 vs G23 plants and (**B**) BTx623 vs G48. Twenty adult SCA were released at the center of a pot containing one plant of each indicated sorghum genotype. Proportion of adult SCA that had settled on each plant combination were monitored after 6 and 24 h post aphid release (n = 15). Asterisks indicate statistically significant differences between the combination (*$P < 0.05$, ** $P < 0.01$) using chi-square test.

difference in the duration of the xylem phase among all three sorghum genotypes (df = 2; $P = 0.4949$; Fig. 5B). Consistent with the no-choice and choice bioassays, a significant difference was observed in the time spent by SCA in the phloem (sieve element) phase (df = 2; $P < 0.0001$; Fig. 5C). The SCA spent significantly more time in the phloem phase of susceptible perennial sorghum genotype (G23), followed by the control and the resistant perennial sorghum genotype (G48). In contrast, the aphids spent significantly less time in the non-probing phase in G23, compared with G48 and BTx623 plants (df = 2; $P = 0.0295$; Fig. 5D). Additionally, we observed that the time to reach the first sieve element phase was similar in the three genotypes (df = 2; $P = 0.145$; Fig. 5E). Supplementary Table 1 shows the mean time spent by SCA for various feeding activities on different perennial sorghum genotypes and the control genotype, BTx623.

Discussion

In this study, we conducted several experiments to identify comparative resistance levels of the perennial sorghum genotypes against SCA infestation. Characterizing and understanding the natural genetic diversity of sorghum is pivotal for better planning of the genetic improvement program[46]. The need for better-performing cultivars with agronomic benefits is crucial for the success of the crop with the changing economic and environmental conditions. Our no-choice assay indicates the varied levels of resistance displayed by the perennial sorghum genotypes to the introduction of apterous aphids (Fig. 1). Aside from hosting significantly fewer aphids than the control genotype (BTx623), some of the genotypes were found to have aphid populations lower than the annual SCA-resistant genotype (Fig. 2). Several studies have screened cereal genotypes against aphid infestation based on these host plant resistance categories. For example, by utilizing genetic variation from different regions of the world, 133 wheat accessions were tested for their resistance levels against *Sitobin miscanthi* infestation using antixenosis resistance screening experiments and choice assay[47]. Similarly, the resistance levels of cotton genotypes were tested against *Aphis gossypii* using no-choice and choice assay[48].

We employed the antibiosis and antixenosis bioassays to understand the plant's defense response of the perennial sorghum genotypes to SCA infestation. Both no-choice and choice bioassay results indicate that resistance levels in G23 and G48 were dictated by both antixenotic and antibiotic-mediated resistance mechanisms (Figs. 1–3). Numerous studies have categorized resistance mechanisms during screening to gain deeper insights into the plant–insect interactions[36,47,49]. Furthermore, EPG helped provide knowledge on the aphid feeding patterns of resistant and susceptible perennial sorghum genotypes. Together, these experiments

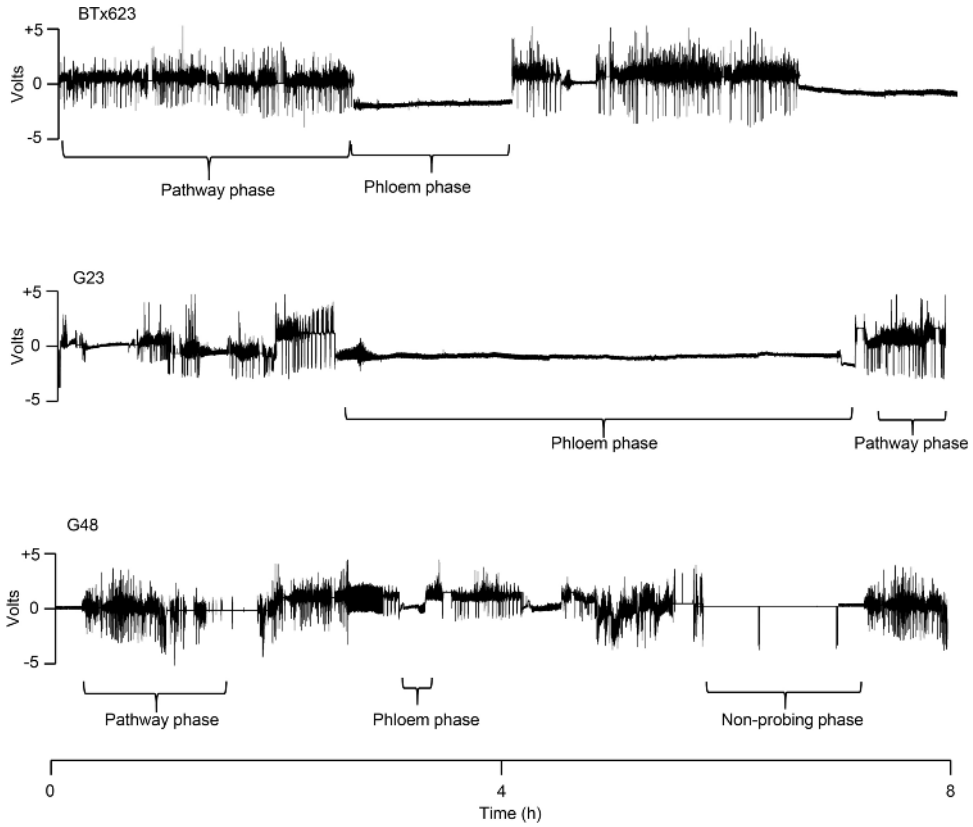

Fig. 4. Representative EPG waveform patterns of SCA feeding on the sorghum BTx623, G23, and G48 plants. The different phases indicate different aphid feeding behavior patterns on the sorghum plants over an 8 h period of EPG recording.

provided valuable insights into the defense mechanisms employed by the perennial sorghum genotypes and how the defenses control SCA stylet movement in various cell layers.

In the no-choice bioassay, we tested the antibiosis level of the perennial sorghum genotypes against SCA infestation. Certain genotypes exhibited high resistance levels such as S1465 > R120D, S1477 > R175A, PR259A ~ Tift126, S1662 > R554B, S14PR > R181, PR230E ~ Tift311, S1852 > 015E, X999 > R485 while others showed low resistance/high susceptibility levels such as PR376 ~ Tift241, PR64A ~ Tift235, PR114A ~ Tift277, PR79 ~ Tift284, PR375 ~ Tift240 genotypes (Figs. 1 and 2). Although specific resistance factors were not investigated, the variation in the resistance levels is attributed to the chemical and/or morphological factors that contributed to difficulties in the survival of aphids[30]. The difference in the aphid populations on different genotypes depicts that feeding on certain genotypes affects SCA's biology and reproduction rate. Host plant quality is one of the major factors affecting antibiosis resistance in plants, accounting for herbivore fitness, performance, and intrinsic plant traits[50,51]. Numerous plant factors can potentially be affecting the aphid fitness of perennial sorghum genotypes. Plant physical structures and chemical repertoire contribute to antibiosis resistance, such as trichomes, toughened cell walls, alkaloids, phenolics, etc[52–56]. Further investigation should be done to better understand the factors impacting aphid resistance in particular genotypes.

To understand the behavioral events driving the variance in resistance levels, we examined host plant selection patterns in two genotypes—one from the cluster of highly resistant genotypes (G48) and one from the highly susceptible group (G23). The strong antixenosis observed in G48, combined with a relatively lower level in G23 compared to the control genotype, suggests that G48 possesses potent insect-deterrent properties, whereas G23 exhibits weaker effects, making it more susceptible. Our results also demonstrated how antixenosis and antibiosis act together in the resistance mechanisms, which have been reported in other studies as well[57,58]. Extensive research has shown how various factors contribute to antixenosis. For example, alterations in flavonoid profiles dictate soybean antixenotic-mediated resistance to *Acyrthosiphon pisum*[59]. The density of leaf trichomes, glandular and non-glandular, regulate resistance levels in wheat against *Sitobion miscanthi*[47]. Trichome density and leaf color mediate preference for feeding and oviposition in soybean against *Spodoptera cosmioides*[60]. Numerous chemical and physical defenses can be credited with providing antixenosis resistance. Occasionally, the difference between the effects of antibiosis and antixenosis regulating insect behavior remains unclear[30,61]. More experiments on leaf characteristics and morphology analysis can help identify the factors that contribute to antixenotic-mediated resistance to aphids in perennial sorghum.

Electrophysiological techniques, such as EPG, are one of the effective methods to explore plant-aphid interactions to localize the plant factors influencing them. We observed the time spent by aphids in each of

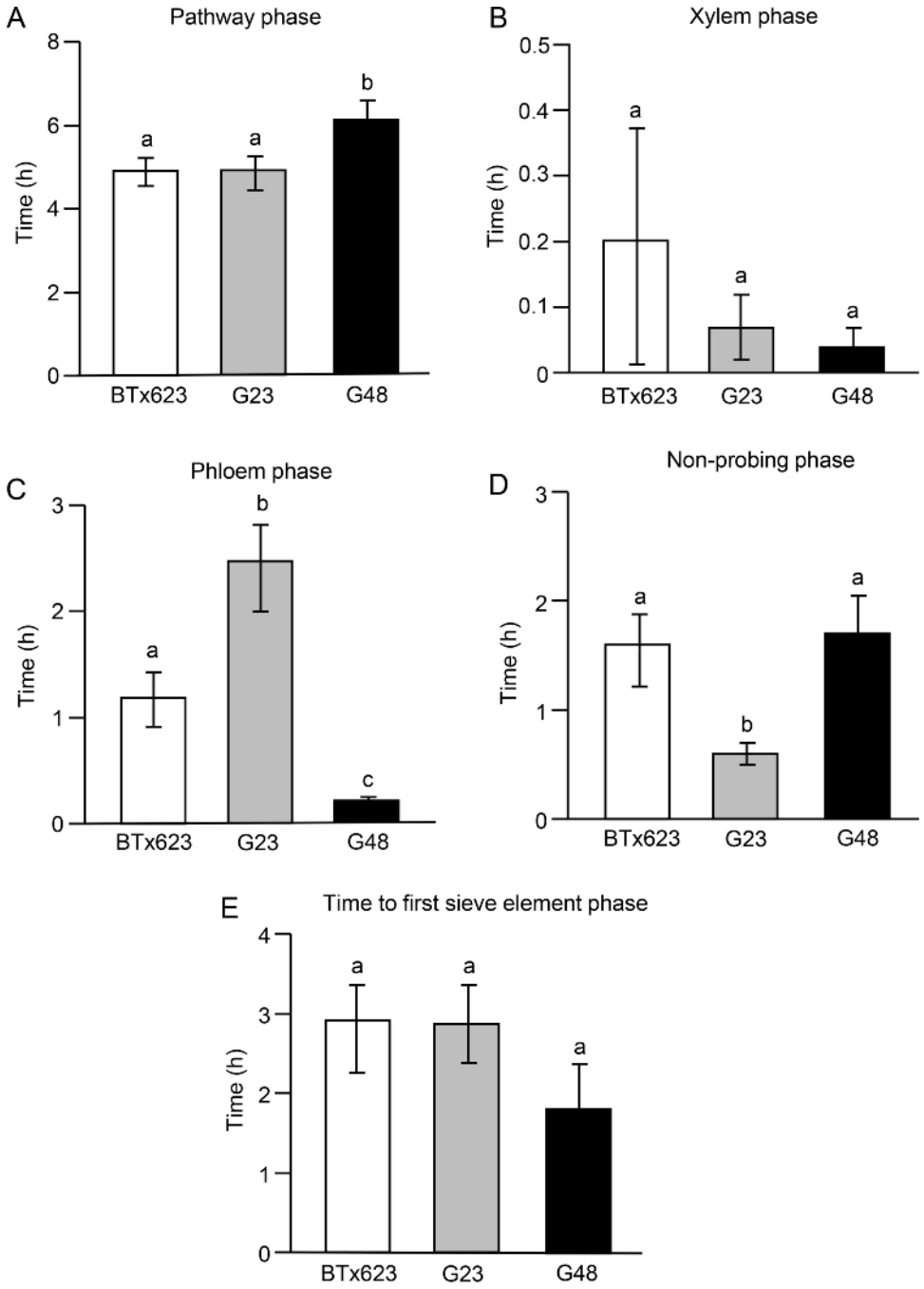

Fig. 5. Total time spent by SCA while feeding on BTx623, G23, and G48 in various feeding phases during an 8 h period of EPG recording (n = 14). (**A**) Pathway phase, (**B**) Xylem phase, (**C**) Phloem phase, (**D**) Non-probing phase, and (**E**) Time to first reach sieve element phase. Bars with different letters represent significant differences from each other ($P < 0.05$; Kruskal–Wallis test). Error bars represent ± SEM.

its characteristic phases in a quantitative manner[38]. EPG recordings revealed differential probing behavior of SCA. Cells were briefly punctured intracellularly in the pathway phase, with the stylets consistently withdrawn and continuing along the intercellular pathway[39]. Aphids spent more time in the pathway phase in G48 than in G23 and BTx623 plants. Additionally, we found that SCA spent significantly more time in the phloem phase in G23, followed by BTx623 and G48 genotypes (Fig. 5). Plant phloem is a rich source of sugars and nutrients and is considered the primary nutrition source for aphids[62,63]. The EPG findings were in agreement with our no-choice results, where we found higher aphid numbers in the perennial sorghum genotype that supported more phloem sap ingestion. Previous studies have shown that the reduced phloem sap ingestion by aphids is strongly associated with enhanced plant resistance. Cowpea aphid (*Aphis craccivora*) spends more time feeding phloem sap and less time expended in the pathway phase in susceptible cowpea cultivars[42]. In Ethiopian mustard, the

phloem ingestion of green peach aphids was significantly less in the aphid-resistant mustard accessions than susceptible ones[35]. Previously, we observed similar patterns in the pathway and phloem phases in SCA feeding on the resistant annual sorghum genotypes[65]. In addition, we found that aphids spent significantly less time in the non-probing phase in G23 than the other two genotypes, which represents the time interval of limited or no stylet movement. Collectively, our results suggest the effectiveness of G23 as a host plant for SCA.

Another notable observation in the aphid feeding behavior was the absence of difference in the time to reach the first sieve element phase among the three sorghum perennial genotypes. The lack of difference in accessing the phloem tissue but differential time spent in the sap ingestion phase suggests the presence of phloem-based defenses in G48. Previously, it was shown that plants can occlude sieve elements by accumulating antinutritive and antibiotic factors in the phloem sap to hinder and control aphid proliferation[64]. Sieve element occlusion can be mediated by phloem proteins (P-proteins) and callose (β-1,3 glucans) deposition in the sieve elements, which blocks stylet's access to phloem[65,66]. Transport and accumulation of secondary metabolites in the phloem sap can also be detrimental to aphids[64]. While feeding on the Brassicaceae family, cabbage aphids sequester and excrete certain types of glucosinolates present in the phloem sap, which affects the aphid host choice[67]. Furthermore, phytohormones can also impact the process of callose deposition in plants. Varsani et al. (2019)[68] demonstrated that OPDA (12-oxo-phytodienoic acid), a precursor of jasmonic acid, signals to induce callose deposition in maize against corn leaf aphid infestation. Furthermore, it was shown that the exogenous application of abscisic acid promoted callose deposition in rice against brown planthoppers[69]. Future experiments should be targeted to explore these pathways to identify the molecular basis of phloem-based defenses in the SCA-resistant perennial sorghum genotype (G48).

In conclusion, the antibiosis resistance screening showed variations in the perennial sorghum genotypes, categorizing them into four clusters. Further, choice and no-choice aphid bioassays support relative differences in SCA resistance in genotypes within these clusters. The EPG results showed a longer duration of pathway phase and shorter duration of phloem sap ingestion with significantly lower aphid preference in G48, indicating strong antixenosis resistance. The information presented in this study can potentially contribute to sorghum breeding programs that aim to utilize wild germplasm to improve environmental sustainability under changing climatic conditions, along with developing novel pest management strategies. Furthermore, the identification of genotypes with natural resistance to economically significant pests can lead to the development of cultivars with broader and substantial impacts. Leveraging natural sorghum germplasm can be highly advantageous for farmers, enabling the simultaneous management of multiple agricultural challenges.

Materials and methods

Plant and insect materials

The reference genotype used in this study was BTx623, with three annual sorghum controls (RTx430, SC265, and SC1345) whose resistance levels were previously characterized[44,70]. The Land Institute, located in Salina, KS, provided the fifty perennial sorghum genotypes. The sorghum plants were grown in the University of Nebraska-Lincoln greenhouse with 16-h light/8-h dark photoperiod under 25 °C and 50–60% humidity for all the experiments. Seeds were grown in cone-tainers in soil mixed with vermiculite and perlite (PRO-MIX BXBIOFUNGICIDE + MYCORRHIZAE, Premier Tech Horticulture Ltd., Canada). Plants were watered daily along with weekly fertigation. Experiments were performed on two-week-old plants[45]. SCA colony was started from a single parthenogenic female collected from SCA-infested sorghum plants at Louisiana State Agricultural Center Dean Lee Research Station, Alexandria, LA, in 2014[45]. The colony is reared on SCA-susceptible BCK60 in the controlled environment conditions described previously[44]. New 3–4-week-old plants were introduced weekly in the colony for aphid propagation. Adult apterous SCA was used in this study.

No-choice bioassay (Antibiosis)

For no-choice bioassay, fifty perennial sorghum genotypes were divided into sets of seventeen-seventeen-sixteen sets to facilitate handling replications at a time. Aphid numbers were recorded for 43 out of 50 perennial sorghum genotypes, while the remaining 7 (G7: TexasHCN_S1662 > 216; G9: S3326 > …PR230F ~ Tift186; G24: S3181 ~ B1 ~ R100H ~ PR294 ~ Tift245; G44: Texas HCN_S1852 > 015E; G45: TexasHCN_X814-201A-PR101C; G46: TexasHCN_X814-201B-R523; G49: TexasHCN_X999-R-348B; G50: S3011-A1D1-D1-Tift128) had germination issues. BTx623 and annual sorghum genotypes were used as controls for no-choice bioassay. Five apterous adult aphids were placed on two-week-old sorghum plants grown in plant growth chambers in previously mentioned environmental conditions. After aphid infestation, plants were covered with tubular plastic cages where ventilation is made possible through organdy fabric circles on the sides and top of the cage. The plants were placed in a completely randomized design among the genotypes and pot-holding trays. Total adult and nymph aphids were counted at 7 days post-infestation (dpi). On 14 dpi, the leaf damage was quantified using a damage rating scale[45,71]. The 1–5 damage scale was used to assess plant damage, where 1 denotes minimal damage (≤ 10%), 2 represents moderate damage (20–39%), 3 indicates substantial damage (40–59%), 4 signifies severe damage (60–79%), and 5 shows extreme damage (≥ 80%) with close to plant death (Fig. S1). The aphid no-choice bioassays had 9–12 replications per genotype.

Choice bioassay (Antixenosis)

The no-choice bioassay assisted in identifying potentially resistant and susceptible perennial sorghum genotypes. We selected two genotypes to test their antixenosis levels of resistance toward SCA infestation. Two-week-old plants, initially grown in Cone-tainers, were transplanted to big pots with dimensions of 10-inch diameter by 9-inch height. We used two combinations PR376 ~ Tift241 (G23) with BTx623 & X999 > R485 (G48) and BTx623 to compare the antixenosis levels of these two genotypes against the reference genotype. Pots were placed equidistantly randomly in the chamber to avoid the effect of orientation and air currents. Twenty apterous adults

were released on the center of the pot on a filter paper on the soil. It was ensured that the filter paper touched the stem of both genotypes. The aphids were then left to choose and settle on either of the genotypes. The number of adult aphids settled on each plant were counted at 6 and 24 h after aphid release for both combinations.

Electrical Penetration Graph (EPG) monitoring of aphid feeding behavior

Two-week-old sorghum plants of PR376 ~ Tift241 (G23), X999 > R485 (G48), and BTx623 were used to monitor the feeding behavior of aphids using EPG analyses. Experimental plan and aphid wiring protocols were followed as described previously[72–74]. Aphids were starved for 1 h in a plastic petri dish before the beginning of the EPG. The dorsal surface of the SCA is adhered to a gold wire that is attached to a brass nail using conductive silver adhesive (insect electrode). A stiff copper wire was inserted in the plant grown in the Cone-tainer, avoiding damage to the roots (plant electrode). The insect and plant electrodes are linked through a GIGA-8 EPG system (W.F. Tjallingii, Wageningen, Netherlands) with a 10^9 Ω resistance amplifier and adjustable plant voltage. The wired insect was placed on the second leaf of the sorghum plant, and readings were recorded for 8 h. All EPG recordings were initiated between 10 and 11 am local time (CST). Each day had a combination of three genotypes for the 8 channels and 14–15 replications were used for each genotype for EPG recordings.

Statistical analysis

For no-choice assays, the aphid counts were normalized against BTx623 (control) for each set. The normalized data were clustered against mean values. For hierarchical clustering, the aphid count and damage rating data were used to make a dendrogram based on the mean Euclidian distance using Ward's method[75]. No-choice assay data were analyzed in R using 'stats' and 'cluster' packages. For choice bioassays, the proportion of aphids choosing replication was used as a numerical entity for analysis. Square root transformed proportions were analyzed using likelihood ratio and χ^2 test to check for any significant difference in choice of aphid settling among genotypes. Choice assay data were analyzed on JMP®, Version 14. SAS Institute Inc. Kruskal–Wallis test was used to analyze EPG data for comparing different feeding parameters and phases between the three genotypes. PROC: Here, NPAR1WAY was used to consider the non-normally distributed data. EPG analysis was done using SAS software (Version 3.81), SAS Institute Inc. For clarity, genotypes are numerically coded, with their corresponding names provided in Table 1.

Data availability

Data is provided within the manuscript or supplementary information files.

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Author contributions

E.K. and J.L. conceived and designed the study. E.K. conducted all the experiments and analyzed the data. P.N., E.M., and S.C. contributed to methods development and provided guidance on experiments. E.K. and J.L. prepared the figures and wrote the draft. All authors contributed to the writing and editing of the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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