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Overexpression of the Sorghum *CCoAOMT* Gene Confers Enhanced Resistance to Sugarcane Aphids

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ABSTRACT

Sorghum (*Sorghum bicolor*) plays a critical role in global agriculture, serving as a staple food source and contributing significantly to various industries. However, sorghum cultivation faces significant challenges, particularly from pests like the sugarcane aphid (SCA), which can cause substantial damage to crops. In this study, we investigated the role of the caffeoyl coenzyme-A *O*-methyltransferase (*CCoAOMT*) gene in sorghum defense against SCA. Feeding by SCA induced the expression of the *SbCCoAOMT* gene, which is involved in the monolignol biosynthesis pathway. Aphid no-choice and choice bioassays revealed that *SbCCoAOMT* overexpression in sorghum resulted in reduced SCA reproduction and decreased aphid settling, respectively, compared to wild-type (RTx430) plants. Furthermore, electrical penetration graph (EPG) studies revealed that *SbCCoAOMT* overexpression restricts aphid feeding from the sieve elements. SCA feeding also induced the accumulation of lignin in sorghum wild-type and *SbCCoAOMT* overexpression plants. Moreover, artificial diet aphid feeding bioassays with hydroxycinnamic acids, ferulic and sinapic acids, showed direct adverse effects on SCA reproduction. Our findings highlight the potential of genetic modification to enhance sorghum resistance to SCA and emphasize the importance of lignin-related genes in plant defense mechanisms. This study offers valuable insights into developing aphid-resistant sorghum varieties and suggests avenues for further research on enhancing plant defenses against biotic stresses.

1 | Introduction

Sorghum (*Sorghum bicolor*) is an economic cereal crop grown for its numerous uses for humans, animals, manufacturing, and agricultural industries. It is a healthy food source for approximately 500 million people in 30 African and Asian countries (Khoddami et al. 2021). Globally, sorghum is a vital crop, with the USA being the largest producer, followed by Nigeria, India, and Mexico (Khalifa and Eltahir 2023). The total global sorghum production exceeds 60 million tons, with more than 50% originating from Africa and Asia (Charyulu et al. 2024). In the USA, sorghum holds a prominent position, with the country being the largest producer and exporter of sorghum, accounting for 20% of world production and almost 80% of world sorghum exports (Mundia et al. 2019; Khalifa and Eltahir 2023). Despite its significance, sorghum production faces challenges worldwide, such as fungal contamination and other invasive pests that constrain increased production levels (Komolong et al. 2003).

In 2013, a massive outbreak of sugarcane aphids (SCA; *Melanaphis sacchari*) occurred in the sorghum-producing regions of the USA (Armstrong et al. 2015; Medina et al. 2017). SCA is a phloem-feeding insect that feeds on the plant sap by inserting its slender stylets, allowing it to feed continuously from phloem tissues, thereby imposing damage to plants (Mou et al. 2023; Thudi et al. 2024; Vasquez et al., 2024). Aphid feeding on sorghum causes stunted growth, yellowing of leaves, and a reduction in grain yield, impacting the overall health and productivity of the crop (Bowling et al. 2016). The ability of SCA to colonize sorghum plants and its impact on grain, forage, and sweet sorghum varieties have been documented, emphasizing the need for effective pest management strategies (Harris-Shultz et al. 2020). Research has focused on understanding SCA's genetic diversity and population structure infesting sorghum, revealing low genetic diversity in aphid populations globally (Nibouche et al. 2014). The genetic mapping of SCA resistance in sorghum has been investigated to develop resistant sorghum hybrids and enhance pest management practices (Cuevas et al. 2022). Commonly used SCA management strategies include the application of insecticides and the cultivation and use of resistant sorghum plants (Harris-Shultz et al. 2020). For example, foliar insecticide applications along with host plant resistance have been suggested as an effective tool in managing SCA in sorghum (Lahiri et al. 2021). Additionally, studies have shown that the appropriate use of host plant resistance is among the safest, cheapest, and most efficient pest management strategies (Sharma et al. 2017). The major mechanisms underlying plant resistance to insects include antibiosis, antixenosis, and tolerance to pests (Painter 1951; Kogan and Ortman 1978). Antibiosis contributes to deleterious physiological effects on insect biology whereas antixenotic-mediated resistance negatively affects insect behavior. Tolerance refers to a plant's ability to endure damage caused by pests without significantly impacting its overall growth and yield (Painter 1951; Koch et al. 2016). Exploration and utilization of host plant resistance have greater potential for improving integrated pest management strategies.

The plant cell wall is the primary defense point, acting as a physical and chemical barrier against various environmental stressors, pathogens, and herbivores (Bacete et al. 2016). Composed of intricate cellulose, hemicellulose, and lignin networks, the cell wall provides structural rigidity, hindering pathogen penetration and deterring herbivore feeding (Voxeur and Höfte 2016). Lignin, an organic polymer in nearly every plant's secondary cell wall, is a complex polymer that provides structural support in plant cell walls. It plays a pivotal role in plant defenses against various environmental stressors, including biotic challenges. The deposition and cross-linking of lignin contribute to reinforcing cell walls, creating a physical barrier that is a deterrent against pathogen invasion and herbivore feeding (Bonawitz and Chapple 2010). Additionally, lignin accumulation is often induced as part of a plant's defense response to herbivory, serving as a crucial component of the plant's arsenal against insect pests (Barros et al. 2016). For example, alterations in the expression of genes related to monolignol biosynthesis modulate plant resistance to aphids (Gallego-Giraldo et al. 2018; Grover et al. 2024). Taken together, these studies provide evidence for the involvement of lignin-related compounds in plant-insect interactions.

The monolignol biosynthetic pathway that produces lignin is a highly conserved and fundamental metabolic route in vascular plants (Barros et al. 2016). This pathway involves the production of three primary monolignols that serve as the building blocks for lignin polymerization, contributing to lignin's structural diversity and functional complexity in plant cell walls (Grover et al. 2024). Phenylalanine ammonia lyase (PAL) initiates the biosynthesis of monolignols, and enzymes such as cinnamate 4-hydroxylase (C4H) and 4-coumarate: CoA ligase (4CL) play a significant role in the synthesis of primary monolignol precursors, namely *p*-coumaroyl, coniferyl, and sinapyl alcohols (Boerjan et al. 2003). The final step in this pathway, which involves the reduction of cinnamyl aldehyde precursors catalyzed by the cinnamyl alcohol dehydrogenase (CAD) enzyme, leads to the formation of hydroxyphenyl (H), guaiacyl (G), and syringyl (S) lignin subunits within the cell wall (Grover et al. 2024; Kundu et al. 2025). Caffeic acid O-methyltransferase (COMT), the penultimate enzyme in the monolignol pathway, facilitates the polymerization of sinapyl alcohol into syringyl (S) lignin polymers through oxidative coupling reactions catalyzed by peroxidases and laccases (Liang et al. 2022; Hoffmann et al. 2020). The monolignol biosynthetic pathway represents a key target for understanding and engineering lignin composition in plants, with implications for enhancing biomass properties and facilitating industrial applications.

Caffeoyl coenzyme-A *O*-methyltransferase (CCoAOMT) is an *S*-adenosyl methionine (SAM)-dependent *O*-methyltransferase that methylates caffeoyl-CoA to generate feruloyl-CoA, an intermediate required for the biosynthesis of both G- and S-lignin subunits (Boerjan et al. 2003). Previously, it was shown that the maize CCoAOMT imparts quantitative resistance to a range of pathogens (Yang et al. 2017). Recently, it was also demonstrated that CCoAOMT enhances resistance to cyst nematodes in soybean plants (Guo et al. 2023). In rice, the trans-CCoAOMT enzyme has been implicated in strengthening the cell wall, thereby activating a disease resistance response (Schmitt et al. 1991). Similarly, in wheat, CCoAOMT has been associated with resistance to Fusarium head blight disease (Yang et al. 2021). However, the role of CCoAOMT in defense against insect pests is unknown. Overexpression of *SbCCoAOMT* leads to phenolic accumulation in sorghum without impacting plant growth and development (Zhong et al. 1998; Tetreault et al. 2018). SbCCoAOMT is a key enzyme involved in the biosynthesis of monolignols and other phenolic compounds in sorghum (Tetreault et al. 2018). The overexpression of *SbCCoAOMT* was revealed to affect the deposition of hydroxycinnamoyl groups in the plant cell walls. Plant phenolic compounds have often been associated with enhanced resistance to insect pests. Thus, this study was aimed at understanding the role of the *SbCCoAOMT* gene in sorghum defense against SCA.

2 | Materials and Methods

2.1 | Plants and Insects

The sorghum lines used in the study were in the RTx430 background, which is the common wild-type pollinator line and sorghum line used for transformation. Two independently

TABLE 1 | RT-qPCR primers used to analyze expression levels of genes from the monolignol biosynthesis pathway where F stands for forward primer sequence 5′ to 3′ and R stands for reverse primer sequence 3′ to 5′.

Gene name	Gene ID	Orientation	Primer sequence (5′ to 3′)
Phenylalanine ammonia lyase (*PAL*)	Sobic.004G220300	F	TCTACGGCGTCACCACGGGG
		R	ACCTCCGACGGCAGCGTGT
4-coumarate:CoA ligase (*4CL*)	Sobic.004G062500	F	CCGAAGGCTCTGAAGTCACCGAG
		R	AGGATCTTGCCGGACGGGTTC
Caffeoyl-CoA *O*-methyltransferase (*CCoAOMT*)	Sobic.010G052200	F	AGATCACCGCCAAGCACCCA
		R	GCGCCGATGAGCTTGATGAGC
Caffeic acid *O*-methyltransferase (*COMT*)	Sobic.007G047300	F	GCTCACCCCTAACGAGGACGG
		R	GCACCGCGTCCTTCAGGTAGTA
Cinnamic alcohol dehydrogenase (*CAD*)	Sobic.004G071000	F	GTGGTGAAGGTGCTCTACTG
		R	CGTTGTAGGACCAGATCTTC
α-tubulin gene (*α-Tub*)	Sobic.001G107200	F	TCGGAAACGCGTGCTGGGAG
		R	AGCATCGTCACCTCCCCCAA

transformed lines, *CCoAOMT*-9a and *CCoAOMT*-28b (ZG 234-3-9A and ZG 234-1-28B; Tetreault et al. 2018), contain a cassette designed to overexpress *SbCCoAOMT* under the control of the *35S* promoter. The seeds were grown in a vermiculite and perlite soil mixture (PRO-MIX BX BIOFUNGICIDE + MYCORRHIZAE, Premier Tech Horticulture Ltd.) in cone-tainers. The plants were raised in a 16:8 h light–dark photoperiod. The plants were watered regularly and fertigated (N:P:K::20:10:20) once a week. Plants at the three-leaf (14–16 days old) stage were used for all the experiments. The SCA (*M. sacchari*) colony was maintained as described previously (Puri, Ikuze, et al. 2023). Briefly, the aphids were maintained on the BCK60 sorghum plant, which is highly susceptible to SCA, at a 16:8 h light–dark photoperiod at 26°C. New plants were introduced into the colony weekly for a continuous supply of aphids.

2.2 | Gene Expression Studies Using RT-qPCR

Sorghum plants were infested with five adult SCAs, which were placed on the leaf opposite the whorl region and were contained in a clip cage. Uninfested plants were also caged, which served as a control. Leaf tissue samples were collected on 6, 24, and 48 h post aphid infestation. Additionally, 7 days SCA-infested and uninfested samples were also collected as a later time point. For leaf tissue collections, aphids were carefully removed from the leaf with a paintbrush, and leaf tissues were flash-frozen in liquid nitrogen and stored at −80°C until further use for RNA extraction. Three biological replicates for all treatments at different time points were used. Around 100 mg of leaf tissues were ground using the 2010 Geno/Grinder (SPEX SamplePrep) for 40 s at 1400 strokes min^{-1}. The homogenized leaf tissue was added to 1 mL of TRI reagent (Sigma-Aldrich). RNA was recovered and purified using the RNA Clean and Concentrator Kit (Zymo Research), and on-column DNase treatment was performed. Extracted total RNA was quantified using a Nanodrop 2000c Spectrophotometer (Thermo Scientific). Complementary DNAs (cDNAs) were synthesized from 1 μg of total RNA using the High-Capacity cDNA reverse transcriptase kit (Applied Biosystems Inc.). cDNAs were diluted to 1:10 before using them for RT-qPCR. The gene expression studies were conducted as described previously (Kundu et al. 2023). The list of gene-specific primers is mentioned in Table 1.

2.3 | Aphid Bioassays

2.3.1 | No-Choice Assay

Each plant was infested with five adult apterous aphids and covered with tubular clear plastic cages. The cages were ventilated with organdy fabric on the sides and top of the cage for proper aeration. All the infested plants were randomly arranged in the racks. After 7 days of infestation, the cages were removed, the total number of aphids, including both nymphs and adults, was counted, and the experiment was replicated twice.

2.3.2 | Choice Assay

For the choice assay, two plants grown in cone-tainers were placed in each pot (10 in. diameter by 9 in. height) while maintaining an equal distance (~5.0 cm) and the cone-tainers were supported with soil. Twenty adult aphids were introduced at the center of the pot on a filter paper placed on the soil, leaving no space between the paper and the sorghum stem for the aphids to escape to the soil. The settled adult aphids on each plant were counted after 6 and 24 h of aphid release. The choice assay was compared between RTx430 and *SbCCoAOMT* overexpression plants. Sixteen plants of each line were used for the bioassays, and the experiment was replicated twice.

2.4 | Monitoring of Aphid Feeding Behavior

The electrical penetration graph (EPG) technique was employed to monitor the feeding behavior of SCAs on sorghum plants (Grover, Cardona, et al. 2022; Grover, Puri, et al. 2022; Grover et al. 2024; Cardona, Grover, Bowman, et al. 2023; Cardona, Grover, Busta, et al. 2023). This method involved simultaneous EPG recordings on eight channels, each dedicated to one aphid per plant. The experiment incorporated a robust design, utilizing at least 12 replicates of aphids to ensure the reliability and statistical significance of the findings. EPG recordings, capturing intricate waveforms representing aphid probing and feeding activities, were analyzed using the EPG analysis software *Stylet*$^+$ developed by EPG Systems in Wageningen, The Netherlands. This software facilitated in-depth insights into the dynamic interactions between SCA and sorghum, allowing for a detailed examination of the aphids' electrical penetration activities, probing duration, and feeding behavior patterns.

2.5 | Artificial Diet Feeding Bioassays

Aphid feeding bioassays were conducted using ferulic, sinapic, and caffeic acids to understand the effect of phenolics on aphid reproduction. A sucrose-based artificial diet was prepared with different concentrations of each of the hydroxycinnamic acids in concentrations of 100 and 400 μM. Twenty percentage of sucrose alone served as the control. Five adult aphids were released in a petri dish and covered with a thin layer of parafilm to facilitate proper insertion of the aphid stylets for feeding as described previously (Grover, Puri, et al. 2022; Grover et al. 2024). The number of aphids in each feeding chamber was counted after 4 days.

2.6 | Lignin Quantification of Sorghum Leaves

The lignin content was determined using the thioglycolic acid (TGA) method as described previously (Kundu et al. 2023). In this procedure, the pellet obtained from 5 mg of freeze-dried leaf powder, extracted with 500 μL pure ethanol, was air-dried overnight at room temperature. Subsequently, the pellet was treated with 500 μL of 2 N HCl and 0.1 mL of TGA at 95°C for 6 h. After washing and resuspension in 500 μL of 1 N NaOH, the mixture was incubated overnight. The resulting supernatant was acidified with 250 μL of concentrated HCl and left overnight at 4°C to facilitate the collection of the lignin thioglycolate pellet. This pellet was dissolved in 500 μL of 1 N NaOH, and the spectrophotometric measurements of lignin were carried out at 280 nm. The lignin content was expressed in absorption values at A280, with 1 N NaOH as the blank.

2.7 | Statistical Analyses

The aphid no-choice data were analyzed using a mixed model, and replications were considered random effects (PROC GLIMMIX, SAS 9.3, SAS Institute). Pairwise comparisons were computed using a *t*-test with an experiment-wise error rate of $\alpha = 0.05$. For choice assays, proportions were taken for the total aphids settled on each plant based on the number of aphids that chose each replication. The aphid proportion data were analyzed following square root transformation to correct for heterogeneous variances. We used generalized linear models (GLM) with a likelihood ratio and Chi-square test to assess the treatment effects on aphid settling behavior. The data were analyzed as entirely randomized. A non-parametric Kruskal–Wallis test was used for EPG data to compare the duration of different feeding parameters/phases between other sorghum plants using the PROC NPAR1WAY procedure, considering the non-normally distributed data. We analyzed the data using Tukey's test for aphid feeding bioassays, gene expression studies, and lignin quantification data.

3 | Results

3.1 | Sorghum Plants Display Elevated Levels of *SbCCoAOMT* Gene Expression Upon SCA Infestation

We initially monitored the expression of the *SbCCoAOMT* gene upon SCA infestation on sorghum wild-type (RTx430) plants. Although no changes in the expression of the *SbCCoAOMT* gene were observed at 6 and 24 h, SCA feeding induced the accumulation of transcripts of the gene encoding for the *SbCCoAOMT* 48 h after SCA infestation compared with SCA-uninfested RTx430 plants (Figure 1A). Furthermore, the expression of *SbCCoAOMT* remained at elevated levels through 7 days of SCA infestation in RTx430 plants (Figure 1B), which suggests SCA feeding-induced *SbCCoAOMT* accumulation in sorghum, potentially contributing to resistance to SCA.

3.2 | *SbCCoAOMT* Overexpression Plants Impact SCA Reproduction and Host Choice

In the no-choice assay, aphids were counted on different sorghum plants after 7 days of SCA infestation. Our results show that *SbCCoAOMT*-9a and *SbCCoAOMT*-28b lines significantly supported fewer aphids compared with RTx430 plants (Figure 2), which suggests that the overexpression of the *SbCCoAOMT* provides an antibiotic-mediated resistance of the plants to SCAs. Representative images of sorghum plants before and after SCA infestation for 7 days are shown in Figure S1. In the choice assay, SCA was allowed to choose between RTx430 and *SbCCoAOMT* overexpression plants. After 6 and 24 h of aphid release, we observed that SCA preferred to settle on RTx430 plants compared to *SbCCoAOMT*-9a and *SbCCoAOMT*-28b lines (Figure 3). These results suggest the overexpression of the *SbCCoAOMT* gene provides antixenotic-mediated resistance to SCA in addition to antibiosis.

3.3 | *SbCCoAOMT* Overexpressed Plants Restrict SCA Feeding From Sieve Elements

The EPG technique is a robust tool for determining the feeding behavior of insects on host plants (Tjallingii 1985; Louis et al. 2012; Nalam et al. 2018). We further performed EPG studies using the RTx430, *SbCCoAOMT*-28b, and *SbCCoAOMT*-9a plants to monitor the feeding behavior of SCA on these sorghum lines. Analysis of different EPG waveforms showed that

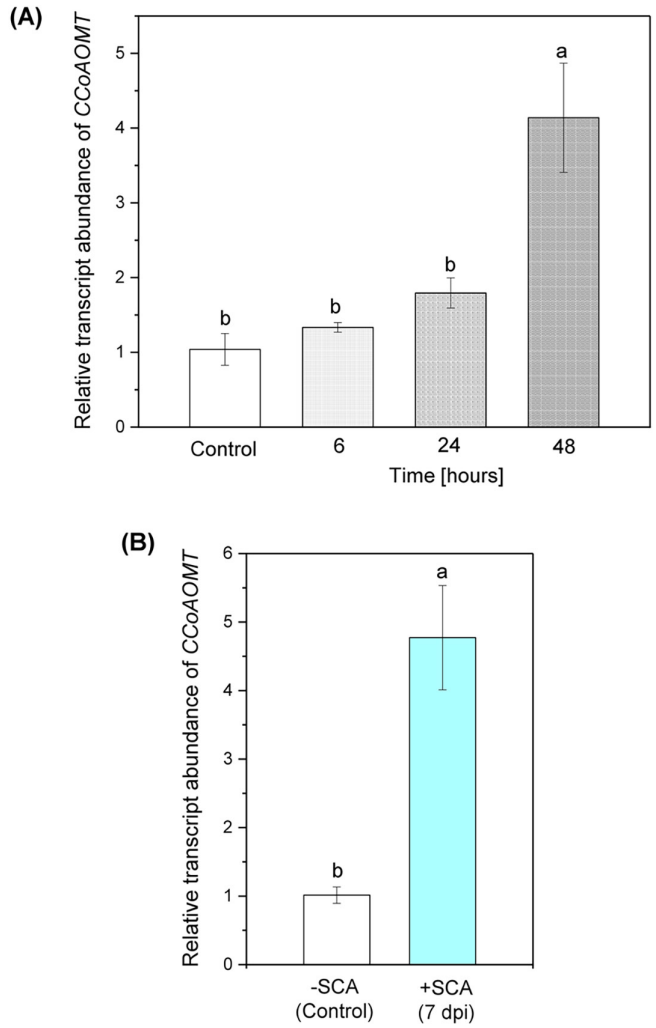

FIGURE 1 | Sugarcane aphid (SCA) feeding triggers the expression of the *SbCCoAOMT* gene. (A) Reverse transcription-quantitative PCR (RT-qPCR) analysis of *CCoAOMT* transcripts in RTx430 plants at early (6, 24, and 48 h) and (B) late time points (7 days post infestation; $n = 4$). SCA-uninfested plants were used as controls. Different letters above the bars indicate values that are significantly different from each other ($p < 0.05$, Tukey's test). Error bars represent ± SEM. dpi, days post infestation.

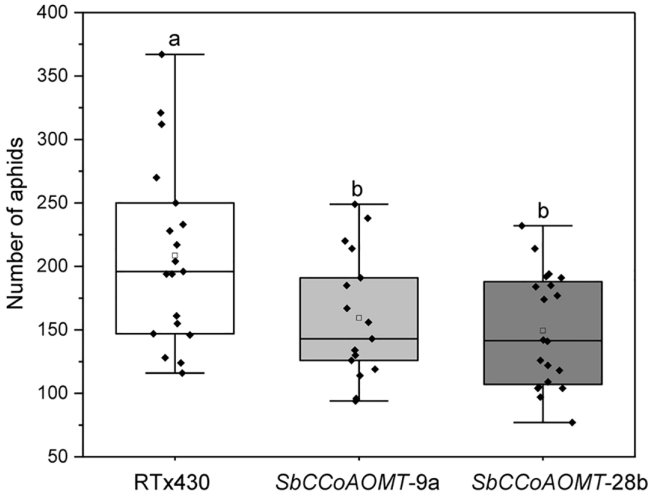

FIGURE 2 | Sorghum *SbCCoAOMT* overexpression lines provide improved resistance to sugarcane aphids (SCA). The total number of SCA (adults and nymphs together) were counted 7 days post infestation of two-week-old sorghum wild-type (WT; RTx430) and *SbCCoAOMT* overexpression lines. Sorghum plants were infested with five adult apterous aphids/plant ($n = 10–13$). Different letters above the bars indicate values that are significantly different from each other ($p < 0.05$, Tukey's test). Error bars represent ± SEM.

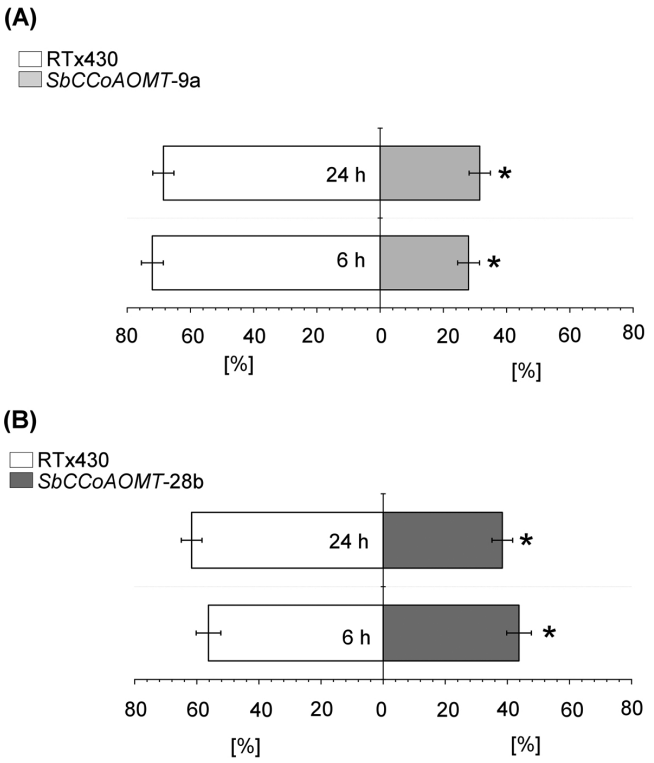

FIGURE 3 | Sugarcane aphids (SCA) preferred to settle on sorghum wild-type (RTx430) plants compared with *SbCCoAOMT* overexpression plants. Choice assay comparison of aphid preference for wild-type (RTx430) vs. *SbCCoAOMT* overexpression lines (A, B) by releasing 20 adult SCAs at the center of a pot containing one plant of each indicated sorghum line. The proportion of adult SCA that had settled on each plant combination was monitored after 6 and 24 h post aphid release ($n = 18$). An asterisk (*) indicates values that are significantly different from each other ($p < 0.05$, χ^2 test). Error bars represent ± SEM.

aphids spent significantly less time in phloem sap ingestion on *SbCCoAOMT*-overexpressed plants compared to RTx430 plants (Figure 4). However, no significant differences were observed in the time spent by SCA in the pathway, xylem, and non-probing phases on these sorghum lines (Figure 4). These results indicate that *SbCCoAOMT* overexpression limited the amount of time the aphids spent feeding from the sieve elements, which suggests the plausible presence of deterring factors in the phloem of these lines by hindering prolonged sap ingestion.

3.4 | SCA Feeding Significantly Increased the Levels of Lignin in Sorghum Wild-Type and *SbCCoAOMT* Overexpression Plants

To further understand the role of monolignol pathway genes, we monitored the relative transcript abundance of the four key

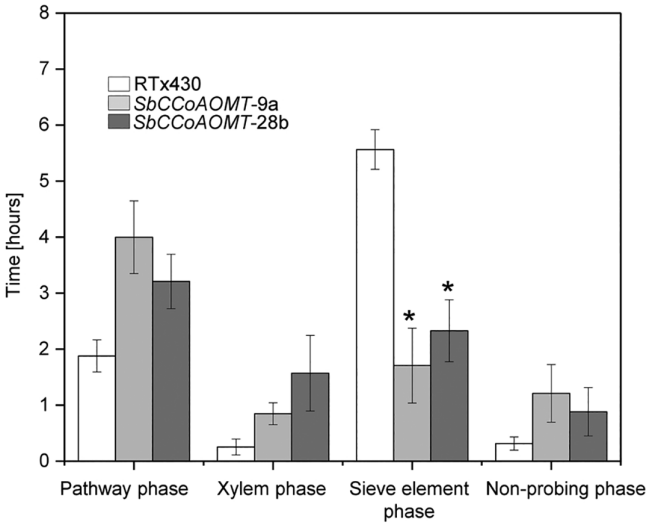

FIGURE 4 | Sorghum *SbCCoAOMT* overexpression lines restrict sugarcane aphid feeding from sieve elements. Electrical penetration graph (EPG) monitoring of mean time spent by SCA for various feeding behavior activities (pathway phase; xylem phase; the total duration of sieve element phase; non-probing phase) in the RTx430 and *SbCCoAOMT* overexpression lines ($n = 15$). Each value is the mean ± SEM. An asterisk (*) represents a significant difference ($p < 0.05$; Kruskal–Wallis test) in the time spent by SCA for the indicated activity on the wild-type (RTx430) and *SbCCoAOMT* overexpression lines.

genes: *PAL*, phenylalanine ammonia lyase; *4CL*, 4-coumarate ligase; *CAD*, cinnamoyl alcohol dehydrogenase; and *COMT*, caffeic acid *O*-methyltransferase, in RTx430, *SbCCoAOMT*-9a, and *SbCCoAOMT*-28b plants 7 days after SCA infestation. SCA-uninfested plants were used as the controls. Although SCA feeding did not alter the expression levels of *PAL* and *CAD* genes (Figure 5A,D,E), significant induction was observed for *4CL* and *COMT* transcript abundance (Figure 5B,C,E). Nearly 3.5- and 4.5-fold upregulation in the transcript of *4CL* was observed in *SbCCoAOMT*-9a and *SbCCoAOMT*-28b lines, respectively, after SCA infestation, whereas RTx430 displayed no changes in the *4CL* expression (Figure 5B,E). The *COMT* transcript was also significantly induced in the *SbCCoAOMT*-28b line as late as 7 days after SCA infestation (Figure 5C,E). Corroborating our transcript data for the lignin biosynthesis genes, the lignin content was also significantly induced in the SCA-fed *SbCCoAOMT*-9a and *SbCCoAOMT*-28b plants 7 days after SCA infestation. Although RTx430 also showed significant accumulation in the lignin content, we observed a significantly higher accumulation in the *SbCCoAOMT*-28b plants (Figure 6). Our data suggest that SCA feeding induced the lignin contents in both RTx430 and *SbCCoAOMT* overexpression plants, but more promisingly in the *SbCCoAOMT*-28b plants.

3.5 | Hydroxycinnamic Acids Have Direct Adverse Effects on SCA Growth and Reproduction

The overexpression of *SbCCoAOMT* led to increased levels of phenolic compounds derived from the monolignol biosynthesis pathway in the plant cell walls (Tetreault et al. 2018). This increase in hydroxycinnamic acids, specifically ferulic, caffeic, and sinapic acids, contributed to increased energy content of the stover and the plant's biomass without any change in growth or lignin composition (Tetreault et al. 2018). Thus, we next tested the influence of hydroxycinnamic acids on SCA growth and development. Our feeding trial bioassays showed that both ferulic and sinapic acids have a direct negative impact on SCA growth and reproduction after 4 days at higher concentrations (400 μM; Figure 7A,B). However, we did not observe any negative effect of caffeic acid on SCA growth and reproduction (Figure 7C).

4 | Discussion

The findings of this study underscore the role of the monolignol pathway-associated gene *SbCCoAOMT* in enhancing sorghum resistance to SCA. As a key secondary cell wall structural component, lignin provides mechanical strength to plant tissues and acts as a physical barrier against various environmental stresses (Sattler and Funnell-Harris 2013). This study shows that aphid infestation triggers the expression of *SbCCoAOMT* involved in the monolignol biosynthesis pathway and an increase in lignin levels after SCA feeding (Figures 1A,B and 6). Previous literature has shown that SCA feeding contributes to the expression of genes involved in the monolignol biosynthesis pathway, particularly in the SCA-resistant line (Puri, Grover, et al. 2023). This increase in expression at early time points suggests that the plant's defense response is activated rapidly upon aphid feeding (Puri, Grover, et al. 2023), and it persists up to 7 days post infestation. Our findings suggest that the overexpression of *SbCCoAOMT* confers antibiosis and antixenosis against aphids, impacting aphid populations and reducing the damage that they cause to plants. The overexpression of *SbCCoAOMT* inhibits the aphids' ability to thrive and deters them from feeding on sorghum plants (Figures 2 and 3). Furthermore, EPG analysis indicated that aphids spend less time in the sieve element phase of *SbCCoAOMT*-overexpressed plants (Figure 4), suggesting phloem-based defenses in sorghum. This disruption in feeding behavior potentially weakens the aphids' ability to uptake the vital nutrient source, thereby limiting their ability to cause damage to plants.

Our no-choice and choice bioassay results demonstrated resistance to SCA in *SbCCoAOMT*-overexpressed plants compared with control plants (Figure 2), signifying the potential of the *SbCCoAOMT* gene in providing defense against SCA. Our findings align with previous research on sorghum-SCA interactions that demonstrated that PAL, an important enzyme acting as the substrate of the monolignol pathway, potentially contributes to sorghum resistance to aphids (Pant and Huang 2022). It was also shown that *SbPAL* interacts with the SA pathway in providing enhanced resistance to SCA (Pant and Huang 2022). Although we did not observe any significant induction of the *PAL* transcript upon SCA feeding, several genes from the phenylpropanoid pathway were significantly induced. However, it remains to be elucidated whether *SbCCoAOMT*-mediated resistance to SCA requires the SA pathway.

Decreased phloem sap-feeding by SCA on *SbCCoAOMT*-overexpressed plants indicated enhanced phloem-based

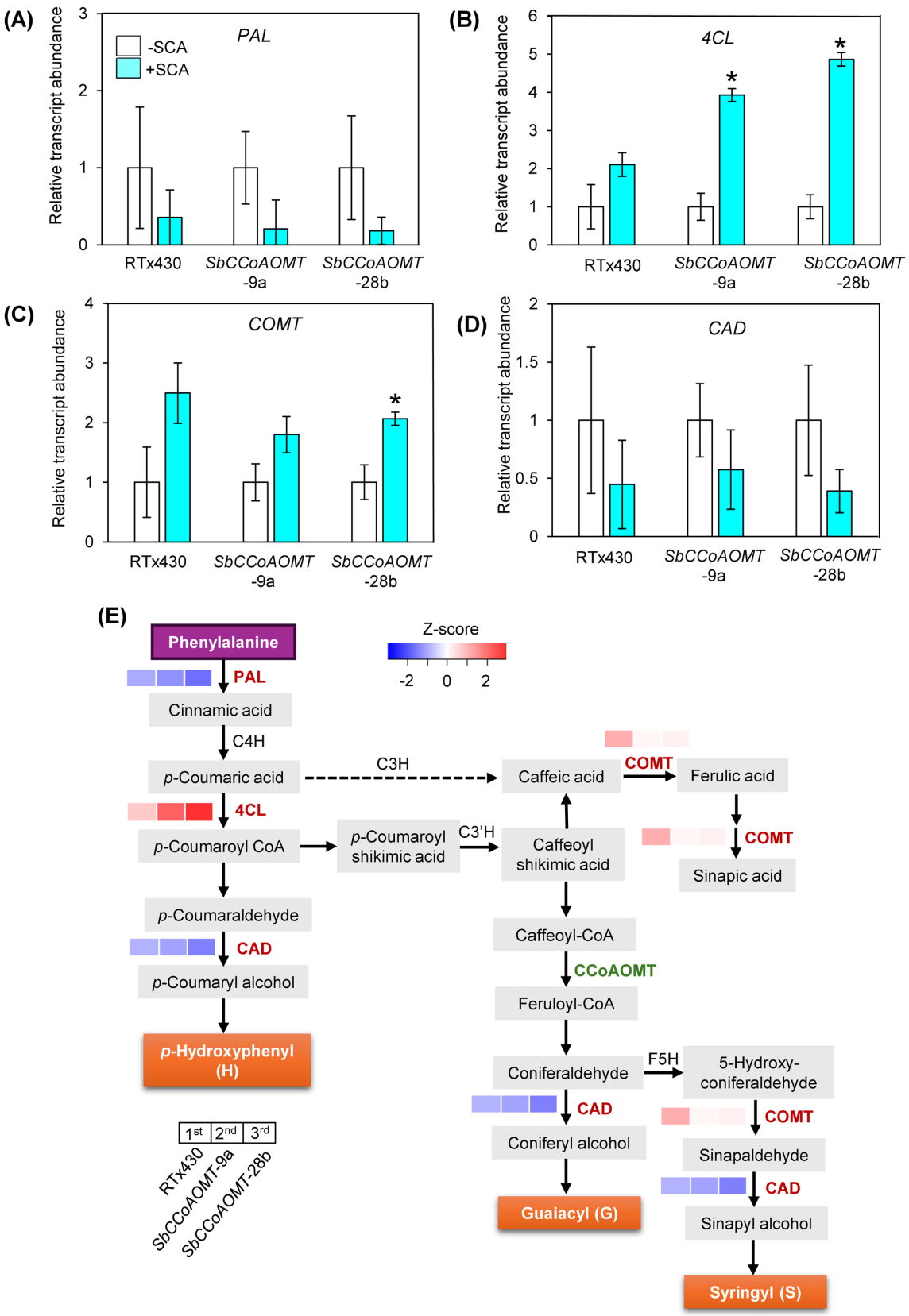

FIGURE 5 | Legend on next page.

resistance to SCA. It is well established that aphids depend on phloem sap for their crucial nutrient source and their access to and consumption of this sap directly affects their survival and reproduction (Cardona, Grover, Busta, et al. 2023). The composition of phloem sap can influence aphid performance and behavior, with variations in nutrient levels affecting aphid feeding rates and preferences (Dinant et al. 2010; Louis et al. 2010; Singh et al. 2011; Nalam et al. 2019; Twayana

FIGURE 5 | Sugarcane aphid (SCA) feeding induced the expression of distinct genes from the monolignol pathway in the *SbCCoAOMT* overexpression lines. SCA feeding altered the relative transcript abundance for the selected genes (A) *PAL*, (B) *4CL*, (C) *COMT*, and (D) *CAD* after 7 days of SCA infestation. SCA-uninfested plants were used as the controls ($n = 4$). An asterisk (*) indicates values that are significantly different compared to their control ($p < 0.05$, Tukey's test). Error bars represent ± SEM. (E) Schematic representation of the monolignol biosynthesis pathway in sorghum representing the expression level of the key genes after 7 days of SCA infestation. 4CL, 4-coumarate:CoA ligase; C3′ H, 4-coumaroyl shikimate 3′-hydroxylase; C3H, 4-coumarate hydroxylase; C4H, cinnamate 4-hydroxylase; CAD, cinnamyl alcohol dehydrogenase; CCoAOMT, caffeoyl-CoA-O-methyltransferase; COMT, Caffeic acid O-methyl transferase; F5H, ferulate 5-hydroxylase; PAL, Phenylalanine ammonia lyase. Broken arrow suggests a potential route in the biosynthesis pathway.

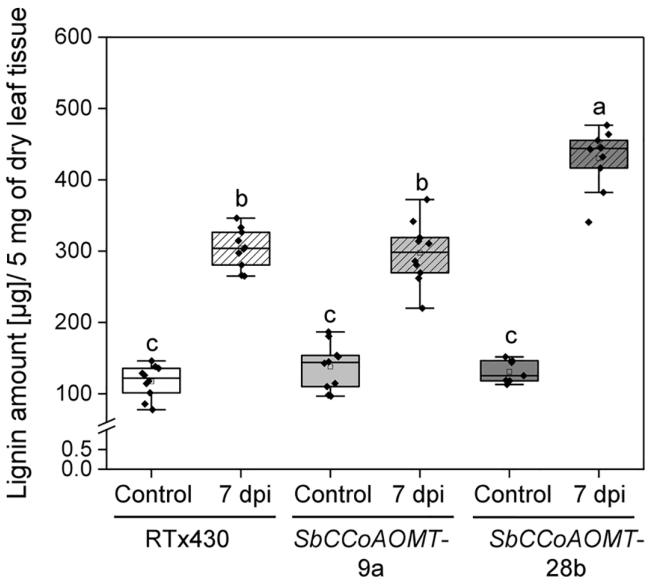

FIGURE 6 | Sugarcane aphid (SCA) feeding induces lignin accumulation in sorghum plants. SCA feeding for 7 days induced the accumulation of lignin in both wild-type (RTx430) and *SbCCoAOMT* overexpression lines. SCA-uninfested plants of same developmental stage as 7 days post infestation (dpi) were used as the controls ($n = 6$). Different letters indicate significant difference relative to each other ($p < 0.05$; Tukey's test). Error bars represent ± SEM.

et al. 2022). Plants can protect themselves from phloem-feeding insects through phloem-based defense mechanisms involving phloem-specific proteins found in the phloem sap. Previously, it was shown that the expression of a functional fragment of harpin protein, Hpa1, in wheat contributed to phloem-based defense against the English grain aphids, *Sitobion avenae* (Fu et al. 2014). Similarly, overexpression of the phloem protein gene *AtPP2-A1* in Arabidopsis significantly diminished phloem sap consumption by the green peach aphid, *Myzus persicae* (Zhang et al. 2011). Although *SbCCoAOMT* overexpression contributes to phloem-based resistance to SCA, the precise mechanism(s) that *SbCCoAOMT* overexpression modulates phloem-based defenses are unknown.

Previously, it has been shown that changes in expression levels of monolignol biosynthesis pathway genes can lead to altered levels of phenylpropanoids in plants (Puri, Grover, et al. 2023; Fornalé et al. 2010). Although we observed significant changes in the expression of *4CL* and *COMT* after SCA infestation in the *SbCCoAOMT* overexpression lines compared with the wild-type plants, the expression of *PAL* and *CAD* were comparable between wild-type and *SbCCoAOMT* overexpression lines before and after SCA infestation (Figure 5A–E). Interestingly, SCA feeding increased the expression of *SbCCoAOMT* (Figure 1) and elevated lignin levels after SCA infestation (Figure 6). *SbCCoAOMT* overexpression resulted in increased accumulation of both soluble and cell wall-bound ferulic and sinapic acids, without affecting lignin concentration or composition. Notably, this enhanced deposition of hydroxycinnamic acids contributed to a higher energy content in the stover (Tetreault et al. 2018). Furthermore, RNA-seq analysis and metabolite profiling revealed that the overall gene expression patterns and metabolite profiles in the overexpression lines were comparable to those observed in wild-type plants (Tetreault et al. 2018). The elevated *SbCCoAOMT* expression after SCA infestation may potentially trigger downstream defense responses in sorghum by inducing the accumulation of lignin and downstream hydroxycinnamic acids. Although we monitored significant accumulation of lignin in the *SbCCoAOMT*-28b line compared with RTx430 and *SbCCoAOMT*-9a lines after SCA infestation, SCA feeding-induced lignin levels were comparable between RTx430 and *SbCCoAOMT*-9a lines (Figure 6), indicating that the plants potentially utilize enhanced lignin accumulation as a generalized plant response to combat aphid attacks. In maize, *ZmCCoAOMT2* was associated with resistance to multiple pathogens (Yang et al. 2017; Ge et al. 2021; Wang et al. 2022). *ZmCCoAOMT2* is also involved in the biosynthesis of lignin and other phenylpropanoid metabolites and the regulation of programmed cell death (Yang et al. 2017; Mu et al. 2021), which is a crucial component of plant defense mechanisms. Whether the sorghum *CCoAOMT* gene functions in a similar way to regulate programmed cell death is unclear. Future work should focus on decoding the underlying mechanisms contributing to *SbCCoAOMT* gene-mediated enhanced resistance to SCAs.

Several studies have suggested the significant role of phenols in plant resistance to aphids. For example, it was demonstrated that *ZmMYB31*, a transcription factor in maize, directly repressed lignin genes, thereby redirecting the phenylpropanoid metabolic flux (Fornalé et al. 2010). This redirection of metabolic pathways potentially enhanced the levels of several phenolic compounds, which are known for their involvement in plant defenses (Mu et al. 2021). Also, flavonoids, a class of phenolic compounds known for their antioxidant properties and potential role in plant defense, have contributed to the resistance mechanism of *Vigna* plants against aphid infestations (Lattanzio et al. 2000). Furthermore, it was shown that phenolic compounds act as antioxidants and are involved in signaling pathways that trigger defense responses against herbivory (Czerniewicz et al. 2017). *SbCCoAOMT* overexpression increased both ferulic and sinapic acids (Tetreault et al. 2018). Our aphid feeding trial bioassays supplemented with ferulic and sinapic acids resulted in elevated

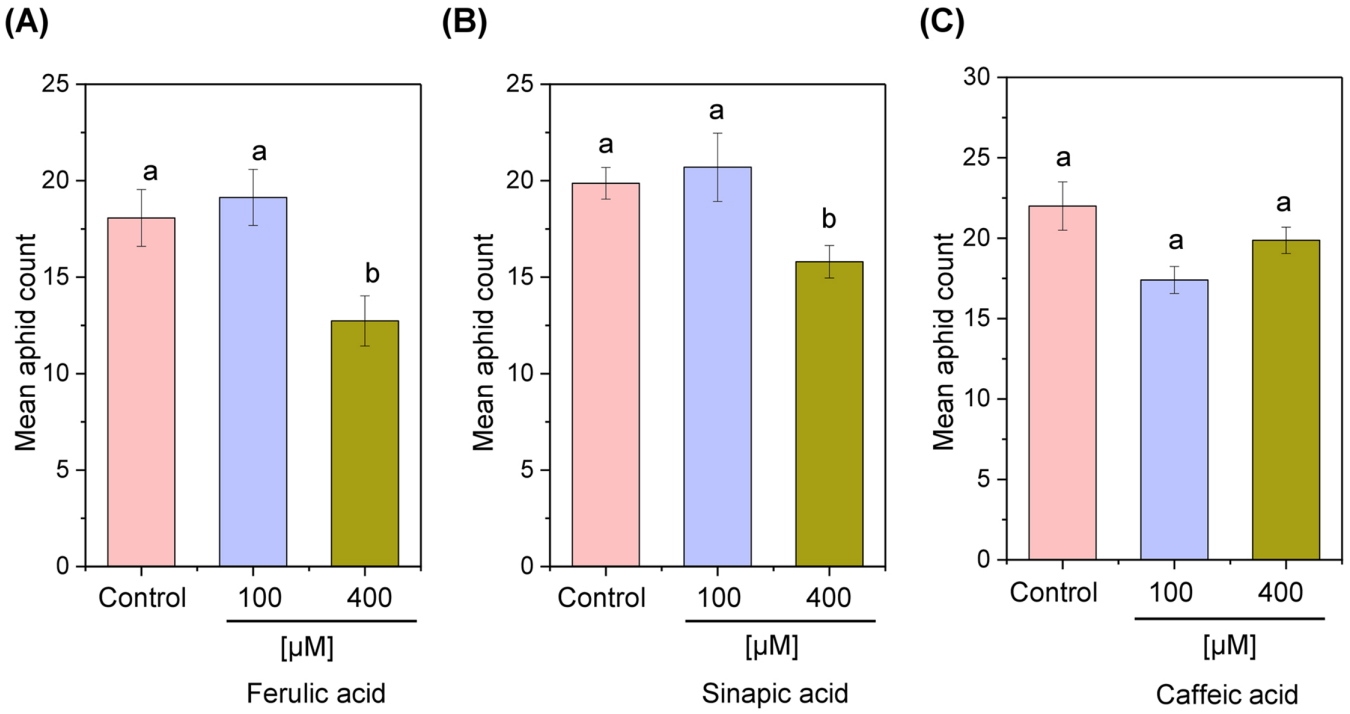

FIGURE 7 | Ferulic and sinapic acids have negative impact on sugarcane aphid (SCA) fecundity. Comparison of SCA numbers on an artificial diet supplemented with two different concentrations of (A) ferulic acid, (B) sinapic acid, and (C) caffeic acid. For feeding trial bioassays, five adult apterous SCA was introduced into each feeding chamber and allowed to feed on the diet. The total numbers of aphids (adults and nymphs) in each chamber were counted after 4 days ($n = 10–15$). Different letters indicate significant difference relative to each other ($p < 0.05$; Tukey's test). Error bars represent ± SEM.

antibiotic activity against SCAs (Figure 7A,B). Collectively, our results suggest that elevated levels of ferulic and sinapic acids due to the overexpression of *SbCCoAOMT* may have a direct negative effect on SCA growth and reproduction. Both sinapic and ferulic acids have been reported to have strong antioxidant properties (Parra et al. 2007; Nithya and Subramanian 2017), which could potentially contribute to antibiotic and antixenotic-mediated sorghum resistance to SCA by modulating the hydrogen peroxide and the superoxide radicals, thereby impacting the flavonoid and other associated pathways. The exact mechanism(s) by which these hydroxycinnamic acids impact SCA physiology need further investigation.

In conclusion, this study sheds light on the importance of the monolignol biosynthesis pathway gene, *SbCCoAOMT*, in enhancing sorghum resistance to SCA. Our findings offer a promising avenue for developing aphid-resistant sorghum varieties through genetic modification. Additionally, the study underscores the need for further research to uncover the intricate molecular mechanisms underlying this enhanced resistance and to explore the potential applications of lignin-related genes in boosting plant defenses against a range of biotic stresses.

Author Contributions

E.I., S.G., and J.L. conceived and designed the study. E.I., S.G., H.P., and P.K. conducted the experiments. J.L. and S.S. contributed reagents and provided guidance on experiments. E.I. and P.K. prepared the figures. E.I. and J.L. prepared the original draft. All authors read, reviewed, and approved the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

References

Armstrong, J. S., W. L. Rooney, G. C. Peterson, R. T. Villanueva, M. J. Brewer, and D. Sekula-Ortiz. 2015. "Sugarcane Aphid (Hemiptera: Aphididae): Host Range and Sorghum Resistance Including Cross-Resistance From Greenbug Sources." *Journal of Economic Entomology* 108: 576–582.

Bacete, L., H. Mélida, E. Miedes, A. Molina, A. Voxeur, and H. Höfte. 2016. "Plant Cell Wall-Mediated Immunity: Cell Wall Changes Trigger Disease Resistance Responses." *Plant Journal* 93, no. 4: 614–636.

Barros, J., J. C. Serrani-Yarce, F. Chen, D. Baxter, B. J. Venables, and R. A. Dixon. 2016. "Role of Bifunctional Ammonia-Lyase in Grass Cell Wall Biosynthesis." *Nature Plants* 2, no. 6: 1–9.

Boerjan, W., J. Ralph, and M. Baucher. 2003. "Lignin Biosynthesis." *Annual Review of Plant Biology* 54: 519–546.

Bonawitz, N. D., and C. Chapple. 2010. "The Genetics of Lignin Biosynthesis: Connecting Genotype to Phenotype." *Annual Review of Genetics* 44: 337–363.

Bowling, R. D., M. J. Brewer, D. L. Kerns, et al. 2016. "Sugarcane Aphid (Hemiptera: Aphididae): A New Pest in Sorghum in North America." *Journal of Integrated Pest Management* 7, no. 1: 12.

Cardona, J. B., S. Grover, M. J. Bowman, et al. 2023. "Sugars and Cuticular Waxes Impact Sugarcane Aphid (*Melanaphis sacchari*) Colonization on Different Developmental Stages of Sorghum." *Plant Science* 330: 111646.

Cardona, J. B., S. Grover, L. Busta, S. E. Sattler, and J. Louis. 2023. "Sorghum Cuticular Waxes Influence Host Plant Selection by Aphids." *Planta* 257, no. 1: 22.

Charyulu, D. K., V. Afari-Sefa, and M. K. Gumma. 2024. "Trends in Global Sorghum Production: Perspectives and Limitations." In *Omics and Biotechnological Approaches for Product Profile-Driven Sorghum Improvement*, 1–19. Springer Nature Singapore.

Cuevas, H. E., J. E. Knoll, K. R. Harris-Shultz, and S. M. Punnuri. 2022. "Genetic Mapping of Sugarcane Aphid Resistance in Sorghum Line SC112-14." *Crop Science* 62, no. 6: 2267–2275.

Czerniewicz, P., H. Sytykiewicz, R. Durak, B. Borowiak-Sobkowiak, and G. Chrzanowski. 2017. "Role of Phenolic Compounds During Antioxidative Responses of Winter Triticale to Aphid and Beetle Attack." *Plant Physiology and Biochemistry* 118: 529–540.

Dinant, S., J. L. Bonnemain, C. Girousse, and J. Kehr. 2010. "Phloem Sap Intricacy and Interplay With Aphid Feeding." *Comptes Rendus Biologies* 333, no. 6–7: 504–515.

Fornalé, S., X. Shi, C. Chai, et al. 2010. "ZmMYB31 Directly Represses Maize Lignin Genes and Redirects the Phenylpropanoid Metabolic Flux." *Plant Journal* 64: 633–644.

Fu, M., M. Xu, T. Zhou, et al. 2014. "Transgenic Expression of a Functional Fragment of Harpin Protein Hpa1 in Wheat Induces the Phloem-Based Defense Against English Grain Aphid." *Journal of Experimental Botany* 65, no. 6: 1439–1453.

Gallego-Giraldo, L., S. Posé, S. Pattathil, et al. 2018. "Elicitors and Defense Gene Induction in Plants With Altered Lignin Compositions." *New Phytologist* 219, no. 4: 1235–1251.

Ge, C., Y. Wang, S. Lu, et al. 2021. "Multi-Omics Analyses Reveal the Regulatory Network and the Function of ZmUGTs in Maize Defense Response." *Frontiers in Plant Science* 12: 738261.

Grover, S., J. B. Cardona, P. Zogli, et al. 2022. "Reprogramming of Sorghum Proteome in Response to Sugarcane Aphid Infestation." *Plant Science* 320: 111289.

Grover, S., D. F. Mou, K. Shrestha, et al. 2024. "Impaired *Brown Midrib12* Function Orchestrates Sorghum Resistance to Aphids via an Auxin Conjugate Indole-3-Acetic Acid–Aspartic Acid." *New Phytologist* 244: 1597–1615.

Grover, S., H. Puri, Z. Xin, S. E. Sattler, and J. Louis. 2022. "Dichotomous Role of Jasmonic Acid in Modulating Sorghum Defense Against Aphids." *Molecular Plant-Microbe Interactions* 35, no. 9: 755–767.

Guo, Z., X. Si, L. Jiao, and D. Liu. 2023. "Cloning and Bioinformatics of CCoAOMT Relating to Resistance of Soybean to Cyst Nematodes." *Fujian Journal of Agricultural Sciences* 38, no. 5: 616–623.

Harris-Shultz, K., J. Knoll, S. Punnuri, E. Niland, and X. Ni. 2020. "Evaluation of Strains of *Beauveria bassiana* and *Isaria fumosorosea* to Control Sugarcane Aphids on Grain Sorghum." *Agrosystems, Geosciences & Environment* 3, no. 1: e20047.

Hoffmann, N., A. Benske, H. Betz, M. Schuetz, and A. L. Samuels. 2020. "Laccases and Peroxidases Co-Localize in Lignified Secondary Cell Walls Throughout Stem Development." *Plant Physiology* 184, no. 2: 806–822.

Khalifa, M., and E. A. Eltahir. 2023. "Assessment of Global Sorghum Production, Tolerance, and Climate Risk." *Frontiers in Sustainable Food Systems* 7: 1184373.

Khoddami, A., V. Messina, K. Vadabalija Venkata, A. Farahnaky, C. L. Blanchard, and T. H. Roberts. 2021. "Sorghum in Foods: Functionality and Potential in Innovative Products." *Critical Reviews in Food Science and Nutrition* 63: 1170–1186.

Koch, K. G., K. Chapman, J. Louis, T. Heng-Moss, and G. Sarath. 2016. "Plant Tolerance: A Unique Approach to Control Hemipteran Pests." *Frontiers in Plant Science* 7: 1363.

Kogan, M., and E. F. Ortman. 1978. "Antixenosis—A New Term Proposed to Define Painter's "Nonpreference" Modality of Resistance." *Bulletin of the Entomological Society of America* 24: 175–176.

Komolong, B., S. Chakraborty, M. Ryley, and D. Yates. 2003. "Ovary Colonization by *Claviceps africana* Is Related to Ergot Resistance in Male-Sterile Sorghum Lines." *Plant Pathology* 52, no. 5: 620–627.

Kundu, P., S. Grover, A. Perez, J. D. Raya Vaca, R. Kariyat, and J. Louis. 2023. "Sorghum Defense Responses to Sequential Attack by Insect Herbivores of Different Feeding Guilds." *Planta* 258, no. 2: 35.

Kundu, P., S. Shinde, S. Grover, S. E. Sattler, and J. Louis. 2025. "Caffeic Acid O-Methyltransferase-Dependent Flavonoid Defenses Promote Sorghum Resistance to Fall Armyworm Infestation." *Plant Physiology* 197, no. 3: kiaf071. https://doi.org/10.1093/plphys/kiaf071.

Lahiri, S., X. Ni, G. D. Buntin, et al. 2021. "Combining Host Plant Resistance and Foliar Insecticide Application to Manage *Melanaphis sacchari* (Hemiptera: Aphididae) in Grain Sorghum." *International Journal of Pest Management* 67, no. 1: 10–19.

Lattanzio, V., S. Arpaia, A. Cardinali, D. Di Venere, and V. Linsalata. 2000. "Role of Endogenous Flavonoids in Resistance Mechanism of *Vigna* to Aphids." *Journal of Agricultural and Food Chemistry* 48, no. 11: 5316–5320.

Liang, S., S. Xu, D. Qu, et al. 2022. "Identification and Functional Analysis of the Caffeic Acid O-Methyltransferase (COMT) Gene Family in Rice (*Oryza sativa* L.)." *International Journal of Molecular Sciences* 23, no. 15: 8491.

Louis, J., K. Lorenc-Kukula, V. Singh, J. Reese, G. Jander, and J. Shah. 2010. "Antibiosis Against the Green Peach Aphid Requires the *Arabidopsis thaliana MYZUS PERSICAE-INDUCED LIPASE1* Gene." *Plant Journal* 64, no. 5: 800–811.

Louis, J., V. Singh, and J. Shah. 2012. "*Arabidopsis thaliana*—Aphid Interaction." *Arabidopsis book/American Society of Plant Biologists* 10: e0159.

Medina, R. F., S. J. Armstrong, and K. Harrison. 2017. "Genetic Population Structure of Sugarcane Aphid, *Melanaphis Sacchari*, in Sorghum, Sugarcane, and Johnsongrass in the Continental USA." *Entomologia Experimentalis et Applicata* 162: 358–365.

Mou, D. F., P. Kundu, L. Pingault, H. Puri, S. Shinde, and J. Louis. 2023. "Monocot Crop-Aphid Interactions: Plant Resilience and Aphid Adaptation." *Current Opinion in Insect Science* 25: 101038.

Mu, X., J. Li, Z. Dai, et al. 2021. "Commonly and Specifically Activated Defense Responses in Maize Disease Lesion Mimic Mutants Revealed by Integrated Transcriptomics and Metabolomics Analysis." *Frontiers in Plant Science* 12: 638792.

Mundia, C. W., S. Secchi, K. Akamani, and G. Wang. 2019. "A Regional Comparison of Factors Affecting Global Sorghum Production: The Case of North America, Asia and Africa's Sahel." *Sustainability* 11, no. 7: 2135.

Nalam, V., J. Louis, M. Patel, and J. Shah. 2018. "Arabidopsis-Green Peach Aphid Interaction: Rearing the Insect, No-Choice and Fecundity Assays, and Electrical Penetration Graph Technique to Study Insect Feeding Behavior." *Bio-Protocol* 8, no. 15: e2950.

Nalam, V., J. Louis, and J. Shah. 2019. "Plant Defense Against Aphids, the Pest Extraordinaire." *Plant Science* 279: 96–107.

Nibouche, S., B. Fartek, S. Mississipi, H. Delatte, B. Reynaud, and L. Costet. 2014. "Low Genetic Diversity in *Melanaphis sacchari* Aphid Populations at the Worldwide Scale." *PLoS One* 9, no. 8: e106067.

Nithya, R., and S. Subramanian. 2017. "Antioxidant Properties of Sinapic Acid: In Vitro and In Vivo Approach." *Asian Journal of Pharmaceutical and Clinical Research* 10, no. 6: 255.

Painter, R. H. 1951. *Insect Resistance in Crop Plants*. Macmillan Company.

Pant, S., and Y. Huang. 2022. "Genome-Wide Studies of *PAL* Genes in Sorghum and Their Responses to Aphid Infestation." *Scientific Reports* 12, no. 1: 22537.

Parra, C. d. l., S. S. Saldivar, and R. H. Liu. 2007. "Effect of Processing on the Phytochemical Profiles and Antioxidant Activity of Corn for Production of Masa, Tortillas, and Tortilla Chips." *Journal of Agricultural and Food Chemistry* 55, no. 10: 4177–4183.

Puri, H., S. Grover, L. Pingault, S. E. Sattler, and J. Louis. 2023. "Temporal Transcriptomic Profiling Elucidates Sorghum Defense Mechanisms Against Sugarcane Aphids." *BMC Genomics* 24, no. 1: 441.

Puri, H., E. Ikuze, J. Ayala, et al. 2023. "Greenbug Feeding-Induced Resistance to Sugarcane Aphids in Sorghum." *Frontiers in Ecology and Evolution* 11: 85.

Sattler, S., and D. Funnell-Harris. 2013. "Modifying Lignin to Improve Bioenergy Feedstocks: Strengthening the Barrier Against Pathogens?" *Frontiers in Plant Science* 4: 70.

Schmitt, D., A. E. Pakusch, and U. Matern. 1991. "Molecular Cloning, Induction and Taxonomic Distribution of Caffeoyl-CoA 3-O-Methyltransferase, an Enzyme Involved in Disease Resistance." *Journal of Biological Chemistry* 266, no. 26: 17416–17423.

Sharma, S., R. Kooner, and R. Arora. 2017. "Insect Pests and Crop Losses." In *Breeding Insect-Resistant Crops Sustainable Agriculture*, 45–66. Springer.

Singh, V., J. Louis, B. G. Ayre, J. C. Reese, and J. Shah. 2011. "*TREHALOSE PHOSPHATE SYNTHASE11*-Dependent Trehalose Metabolism Promotes *Arabidopsis thaliana* Defense Against the Phloem-Feeding Insect *Myzus persicae*." *Plant Journal* 67, no. 1: 94–104.

Tetreault, H. M., E. D. Scully, T. Gries, et al. 2018. "Overexpression of the *Sorghum bicolor SbCCoAOMT* Alters Cell Wall Associated Hydroxycinnamoyl Groups." *PLoS One* 13, no. 10: e0204153. https://doi.org/10.1371/journal.pone.0204153.

Thudi, M., M. S. Reddy, Y. D. Naik, et al. 2024. "Invasive Sorghum Aphid: A Decade of Research on Deciphering Plant Resistance Mechanisms and Novel Approaches in Breeding for Sorghum Resistance to Aphids." *Crop Science* 64: 2436–2458. https://doi.org/10.1002/csc2.21301.

Tjallingii, W. F. 1985. "Electrical Nature of Recorded Signals During Stylet Penetration by Aphids." *Entomologia Experimentalis et Applicata* 38, no. 2: 177–186.

Twayana, M., A. M. Girija, V. Mohan, and J. Shah. 2022. "Phloem: At the Center of Action in Plant Defense Against Aphids." *Journal of Plant Physiology* 273: 153695.

Vasquez, A., J. Belsky, N. Khanal, et al. 2024. "*Melanaphis sacchari/sorghi* Complex: Current Status, Challenges and Integrated Strategies for Managing the Invasive Sap-Feeding Insect Pest of Sorghum." *Pest Management Science* 81, no. 5: 2427–2441. https://doi.org/10.1002/ps.8291.

Voxeur, A., and H. Höfte. 2016. "Cell Wall Integrity Signaling in Plants: "to Grow or Not to Grow That's the Question"." *Glycobiology* 26, no. 9: 950–960.

Wang, Y., T. Li, Z. Sun, et al. 2022. "Comparative Transcriptome Meta-Analysis Reveals a Set of Genes Involved in the Responses to Multiple Pathogens in Maize." *Frontiers in Plant Science* 13: 971371.

Yang, G., W. Pan, R. Zhang, et al. 2021. "Genome-Wide Identification and Characterization of Caffeoyl-Coenzyme A O-Methyltransferase Genes Related to the Fusarium Head Blight Response in Wheat." *BMC Genomics* 22: 504.

Yang, Q., Y. He, M. K. Kabahuma, et al. 2017. "A Gene Encoding Maize Caffeoyl-CoA *O*-Methyltransferase Confers Quantitative Resistance to Multiple Pathogens." *Nature Genetics* 49, no. 9: 1364–1372.

Zhang, C., H. Shi, L. Chen, et al. 2011. "Harpin-Induced Expression and Transgenic Overexpression of the Phloem Protein Gene *AtPP2-A1* in Arabidopsis Repress Phloem Feeding of the Green Peach Aphid *Myzus persicae*." *BMC Plant Biology* 11: 1–19.

Zhong, R., W. H. Morrison III, J. Negrel, and Z. H. Ye. 1998. "Dual Methylation Pathways in Lignin Biosynthesis." *Plant Cell Reports* 10, no. 12: 2033–2045.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.