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Dual role of $KHCO_3$ priming: reduces seed-borne pathogens on sorghum seeds and promotes seed germination

Shuai Dong[1,2,3,4†], Keqin Peng[1,2,4†], Qiyuan Liu[1,2,4], Min Zhang[1,2,4], Yan Luo[1,2,4] and Zhen Hua Li[1,2,4*]

Abstract

Background Seed-borne diseases have seriously affected the sustainability of sorghum cultivation in China as the demand for organic products in the winemaking industry has limited the use of chemical fungicides.

Results This study conducted a comprehensive analysis of fungal diversity in sorghum seeds from three major growing regions in Guizhou Province. Using a combination of traditional tissue separation and high-throughput sequencing, we identified *Colletotrichum*, *Fusarium*, *Cladosporium*, and *Alternaria* as dominant fungi. Pathogenicity tests revealed that strains GD202206, GD202219, and GD202242 were pathogenic and were identified as *C. sublineola* through morphological and multi-locus phylogeny analysis (ITS、*CAPDH*、*ACT*、*CHS-1* and *TUB2*). 16 fungicides for seed priming experiments with sorghum seeds, the results indicated that priming with $KHCO_3$ significantly enhanced germination of the sorghum seeds, with both indoor and outdoor emergence rates notably higher. Analysis of the fungal changes before and after $KHCO_3$ priming revealed a significant reduction in the abundance of the *Colletotrichum* genus. Additionally, $KHCO_3$ altered the community structure of fungi within the sorghum seeds, reducing population richness. Inter-generic relationships were rebalanced, with antagonism decreased and synergy increased following $KHCO_3$ treatment. Non-target metabolomic analysis indicated that $KHCO_3$ enhances sorghum seed germination via the phenylalanine and flavonoid pathways and exhibits antifungal properties through the cyanoamino acid metabolic pathway.

Conclusion This study identified *C. sublineola* as the primary pathogenic fungus carried by sorghum seeds. $KHCO_3$ treatment has a dual effect on sorghum seeds: on one hand, it suppresses pathogen transmission by reducing the abundance of the *Colletotrichum* genus; on the other hand, it promotes germination and seedling emergence, thereby enhancing both germination and emergence rates.

Keywords Sorghum, Seed priming, *C. sublineola*, Potassium bicarbonate, Seed germination

†Shuai Dong and Keqin Peng are co-first authors and contributed equally to this work.

*Correspondence:
Zhen Hua Li
zhli3@gzu.edu.cn
Full list of author information is available at the end of the article

Background

Sorghum (*Sorghum bicolor* L. Moench) ranks as the fifth most widely consumed cereal globally and is cultivated across over 90 countries [1, 2]. Valued for its exceptional adaptability, sorghum is grown not only in fertile soils but also in adverse conditions such as saline-alkali lands that are unsuitable for many other crops [3]. It possesses a range of uses, serving as a food source for humans and animals alike, and as a raw material in the production of feed, wine, and alternative energy sources [4, 5]. Anthracnose, a common foliar disease, is a widespread concern in sorghum cultivation, particularly in regions like South America, North America, East Asia, and East Africa, where it leads to considerable yield losses each year [6, 7]

The global seed trade has inadvertently facilitated the rapid spread of seed-borne pathogens, which have become a prevalent vector for disease transmission [8]. In several crops, anthracnose has been attributed to the same seed-borne pathogenic species. For example, *Colletotrichum gloeosporioides* is a causative agent of anthracnose in strawberries [9], coffee [10], and onions [11], while *C. truncatum* is known to induce the disease in soybeans [12], lima beans [13], cotton, and various other crops [14]. Furthermore, anthracnose in a single crop can be the result of multiple seed-borne pathogen species. Pepper anthracnose, for instance, has been reported to be caused by C. capsici [15] and *C. acutatum* [16]. Similarly, *C. coccodes*, *C. gossypii*, *C. nymphaeae*, and *C. lindemuthianum* have been identified as pathogens responsible for anthracnose in tomato [17], cotton [18] celery [19], and dry beans [14], respectively.

Seed enhancement technologies are frequently utilised to manage seed-borne anthracnose, as they effectively curtail the propagation of pathogens from seeds to seedlings and mature plants. Synthetic chemicals are the most prevalent and potent agents selected for pathogen control. Coating legume seeds with chemical polymers and chemical pesticides (fludioxonil + mefenoxan and thiabendazole + thiram) improves seed germination and reduces the spread of anthracnose caused by *C.lindemuthianum* [20] and *C.truncatum* [12], Similarly, coating pepper seeds with chitosan polymer has been shown to decrease infection rates by *C. gloeosporioides* [21].Nonetheless, the extensive application of these chemicals may give rise to potential environmental concerns, such as the development of fungicide resistance in certain pathogens [22].

Biological agents or physical treatment techniques are taken as alternatives to chemical products, as they are also effective in sterilizing the pathogen of seed-borne anthracnose. Biopriming of pepper seeds with *Trichoderma harzianum* or *Rhizobacteria Bacillus* reduces seed-borne anthracnose caused by *C. truncatum* [23, 24]. Biopriming of soybean seeds with *Pseudomonas aeruginosa* or *T. harzianum* reduces seed-borne damping-off caused by *C. truncatum* [25]. Biopriming of sorghum seeds with *Trichoderma asperellum* and *T. harzianum* reduces seed-borne anthracnose caused by *C. sublineolum* [22, 26]. Solar UV-B radiation of lupin seeds reduces the incidence of seed-borne anthracnose caused by C. acutatum [16, 27]. Microwave radiation of dry bean and eucalyptus seed was effective in preventing the incidence of seed-borne anthracnose caused by *C. lindemuthianum* and *C. kahawae*, respectively [28, 29]. Laser irradiation of soybean seeds eliminates seed-borne anthracnose caused by *C. truncatum* [25].

In recent times, inorganic salts have emerged as biocompatible alternatives to fungicides in the management of plant pathogens. They are cost-effective, carry a low risk of phytotoxicity, and offer a valuable strategy for combating resistant pathogens. Ammonium bicarbonate has shown promise as an alternative to chemical fungicides in managing Ilyonectria root rot in kiwifruit [30]. Similarly, potassium bicarbonate ($KHCO_3$) has been effectively used to control grey mould in kiwifruit and tomato under various semi-commercial conditions [31, 32]. However, the application of inorganic salts like $KHCO_3$ for controlling seed-borne diseases remains relatively underexplored. In this study, we will screen environmentally friendly antifungal agents to prevent seed-borne anthracnose, enhance seed germination rates in both indoor and outdoor environments, and analyze the regulatory effects of antifungal agents on the fungal community structure within seeds. The study provides a new perspective on the biological control of seed-borne anthracnose.

Results

Diversity analysis of fungi carried by sorghum seeds

During sorghum seed germination experiments conducted in the laboratory, it was observed that a significant portion of the seeds were contaminated with fungi, which severely compromised the germination rate and resulted in seedling infections (Fig. 1). To ascertain the diversity of fungi associated with sorghum seeds, a comparative study was undertaken using both traditional tissue separation techniques and high-throughput sequencing (HTS) methodologies. This study involved the collection of sorghum seeds from three principal regions in Guizhou Province. Subsequently, the fungi present on these seeds were then subjected to a diversity analysis. The findings from the tissue separation method identified a total of 28 fungal genera. *Fusarium* was identified as the predominant genus in both Renhuai (Fig. 2RI) and Guiyang (Fig. 2GI) regions, whereas *Colletotrichum* was found to be the dominant

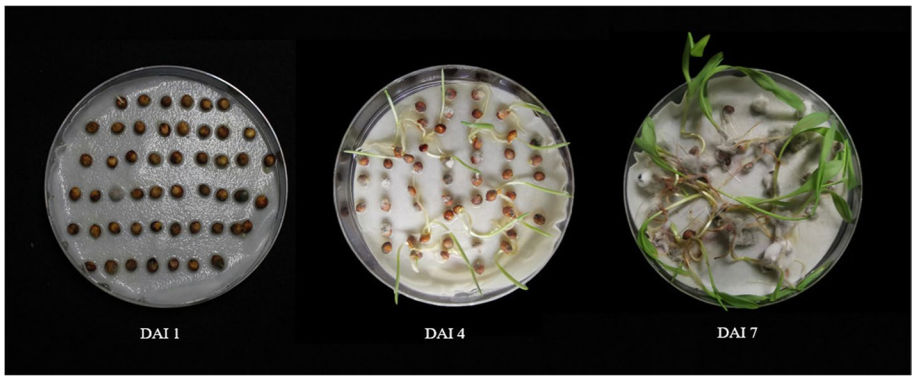

Fig. 1 Rot symptoms appear during germination and post-germination development of infected seeds

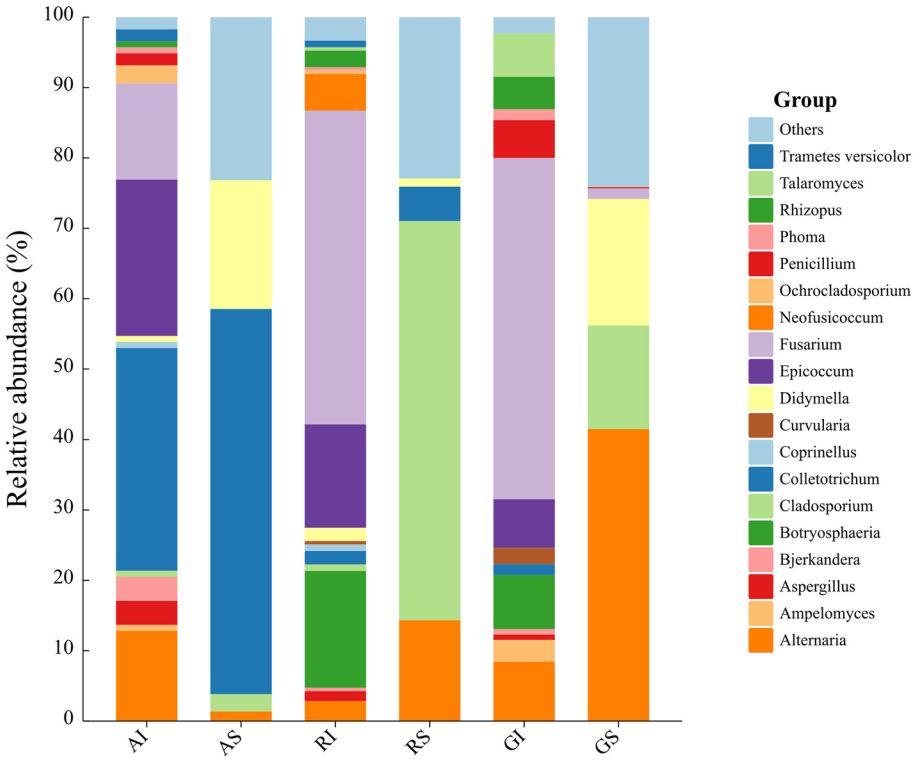

Fig. 2 Fungal diversity of naturally infected seeds in sorghum. By ITS fungal diversity sequencing (S) and single colony isolation techniques (I), fungal genera were identified in seeds harvested from Anlong (AS and AI), Renhuai (RS and RI) and Guiyang (GS and GI), respectively. The vertical axis indicates the percentage of each genus in the total genera

genus in Anlong (Fig. 2AI). Furthermore, the genera *Colletotrichum*, *Fusarium*, *Epicoecum*, and *Alternaria* were observed to be present across all three regions. High-throughput sequencing analysis yielded insights into the relative proportions of different fungi, with *Colletotrichum* being notably prevalent in the Anlong region (Fig. 2AS), *Cladosporium* emerging as the dominant genus in Renhuai (Fig. 2RS), and *Alternaria* being the leading genus in Guiyang (Fig. 2GS). Discrepancies in fungal richness were observed between the two methods; however, upon synthesis of the data, it became evident that *Colletotrichum*, *Fusarium*, *Cladosporium*, and *Alternaria* were the top three genera in terms of fungal richness carried by the seeds across all three locations.

Pathogenicity testing and identification of pathogens in fungi carried by sorghum seeds

We will conduct pathogenicity experiments on fungal strains obtained through the tissue dissection method to identify pathogenic fungi. Given the substantial number of isolates acquired, we have preliminarily classified them based on morphological characteristics and identification results from the internal transcribed spacer (ITS) region. Subsequently, from each genus with strains exhibiting morphological similarities, we will randomly select three strains for pathogenicity testing. The pathogenicity results revealed that only the strains GD202206, GD202219, and GD202242, which share similar morphological characteristics, were found to be pathogenic. In the pathogenicity assays, the pathogenic mycelium initially extended from the seed along the hypocotyl of the seedlings. By day 5, necrosis and wilting of the hypocotyl were observed, followed by the mycelium's invasion into the seedling's root system, resulting in root necrosis (Fig. 3B-E). In contrast, the control group exhibited no signs of disease after the same duration (Fig. 3A). Moreover, these strains also demonstrated pathogenicity towards sorghum leaf tissue (Fig. 3F). Fungal re-isolates from the diseased seeds and leaves were found to be morphologically consistent with the originally inoculated fungi. These findings confirm that the three aforementioned strains are indeed pathogens of sorghum.

The colony (GD202206) of the strain on PDA appears white or off-white, with dense aerial mycelium and even growth. The colony is white and fluffy on the front, with a neat and smooth edge. On the back, the colony has an orange or orange-yellow halo, which deepens over time, turning into orange-yellow or orange-brown (Fig. 4A). Under microscopic observation, distinct pycnidia can be observed at the site of disease occurrence, along with an abundance of setae (Fig. 4B). The conidia are sickle-shaped, germinating from one end, smooth-walled, hyaline, and exhibit no significant size variation (Fig. 4C-F). They are characterized by a consistent morphology and ranged in size from

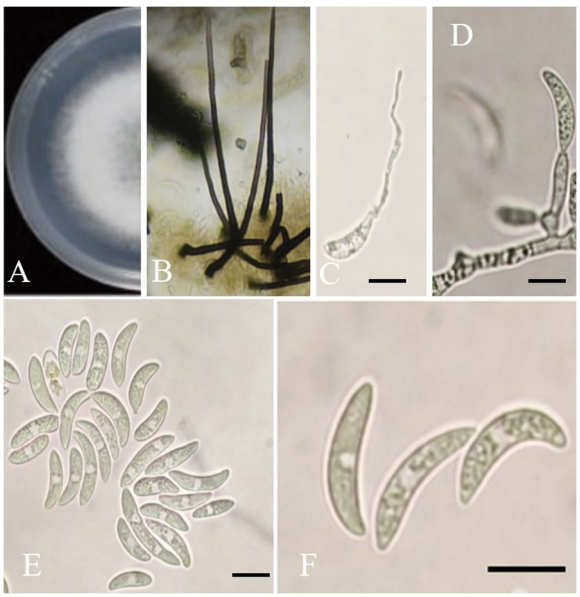

Fig. 4 Morphological characteristics. **A**-**B**: Morphological features of GD202206 on PDA; **C**: Setae; **D**: Unilateral germination of spores; **E**–**F**: Conidia

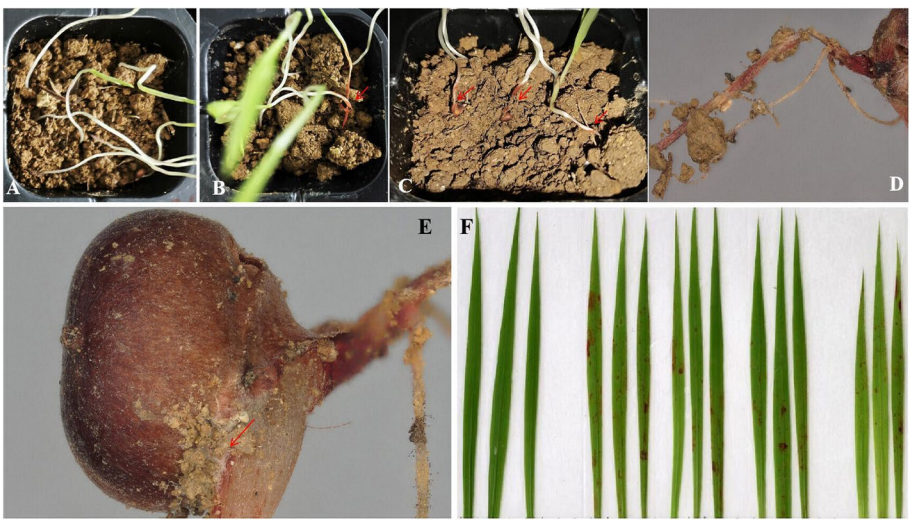

Fig. 3 Pathogenicity test. **A**: Control (CK); **B**-**C**: Seedling stems and seeds show rot after inoculation with the pathogen for 5 days; **D**-**E**: Symptoms on seeds and roots; **F**: Symptoms on leaves

15.46–28.19 μm × 3.75–7.95 μm ($n = 50$). Its morphology is consistent with that of *C. sublineola* YB-NX-1.

Based on the genes ITS, *CAPDH*, *ACT*, *CHS-1*, and *TUB2*, using *Monilochaetes infuscans* CBS 869.96 as an outgroup, a multi-gene phylogenetic tree was constructed using the Maximum Likelihood (ML) method. The multi-gene phylogenetic tree reveals that our three strains, GD202206, GD202219, and GD202242, cluster with *C. sublineola* on a single branch, with a 100% support rate. Therefore, based on the morphological and phylogenetic tree analysis results, the strains were identified as *C. sublineola* (Fig. 5).

Bioeffectiveness of $KHCO_3$ on seed-borne diseases

The effect of priming was compared among 16 fungicides, all of which are biocompatible products officially approved for commercial trade in China. The appropriate concentrations of each product were initially set based on literature references for seed priming treatments. For each reagent, different concentration gradients were established, and laboratory seed priming experiments

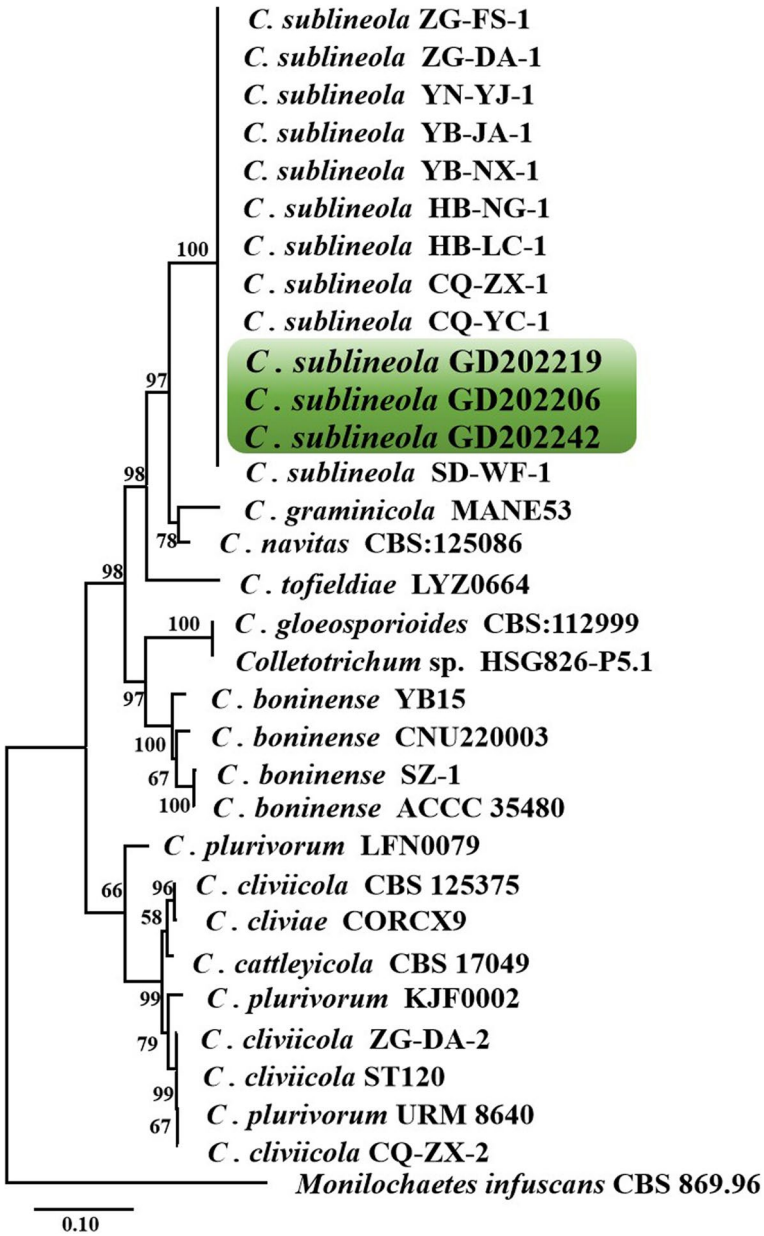

Fig. 5 A multi-gene phylogenetic tree constructed based on ITS, *CAPDH*, *ACT*, *CHS-1*, and *TUB2* sequences

were conducted to evaluate the germination rates at various concentrations. The optimal concentration for each treatment was then confirmed through pot experiments. After priming with $KHCO_3$, the laboratory germination rate was significantly higher than the control, although there was no significant difference compared to most chemical fungicides (Fig. 6). However, multiple outdoor experiments confirmed that $KHCO_3$ priming significantly increased sorghum seedling emergence (Fig. S1).

The differences in fungal genera carried by seeds before and after $KHCO_3$ priming were compared using high-throughput sequencing. The PCoA results showed significant differences in the seed-borne fungal community composition before and after treatment at the three different growing locations. Within the same region, sorghum seed samples treated with the same method exhibited higher similarity in their fungal communities. This indicates that the sequencing samples are reproducible and have an acceptable confidence interval (Fig. S2). Initially, the $KHCO_3$ priming notably altered the abundance of several fungal genera, with a reduction observed in 21, 20, and 14 genera and an increase in 9, 3, and 2 genera in the infected seeds from the respective regions (Fig. 7). Specifically, a concurrent decrease was noted in 12, 11, and 9 genera in Anlong-Renhuai, Renhuai-Guiyang, and Anlong-Guiyang, respectively, while an increase was observed in only one genus across Anlong-Guiyang. Concurrently, 6 and 0 genera were found to decrease and increase, respectively, across all three regions (Fig. 7). Notably, the abundance of *Pseudozyma*, *Papiliotrema*, *Sporobolomyces*, *Cercospora*, *Hannaella*, and *Colletobasidium* was significantly higher in infected seeds compared to those subjected to $KHCO_3$ priming, with a consistent pattern observed across seeds from all three locations (Fig. 8).

Furthermore, $KHCO_3$ priming markedly modified the interrelationships among the genera (Fig. 9). In the non-primed seeds, almost every genus exhibited positive or negative correlations with multiple other genera (Fig. 9A), whereas such negative correlations were substantially diminished in the $KHCO_3$-primed seeds (Fig. 9B). *Ophiosphaerella* and *Lophiostoma* showed no correlation with other genera in the non-primed seeds (Fig. 9A), whereas *Hygrocybe*, *Penicillium*, *Mortierella*, *Paramycosphaerella*, *Phyllosticta*, *Ceratobasidium*, and *Fusarium* were found to be independent of other genera following $KHCO_3$ priming (Fig. 9B). *Colletotrichum* displayed negative correlations with 12 other genera in the non-primed seeds, with only one such correlation persisting in the $KHCO_3$-primed seeds. Most importantly, the abundance of *Colletotrichum* was significantly reduced by $KHCO_3$ priming in infected seeds harvested from all three regions, and

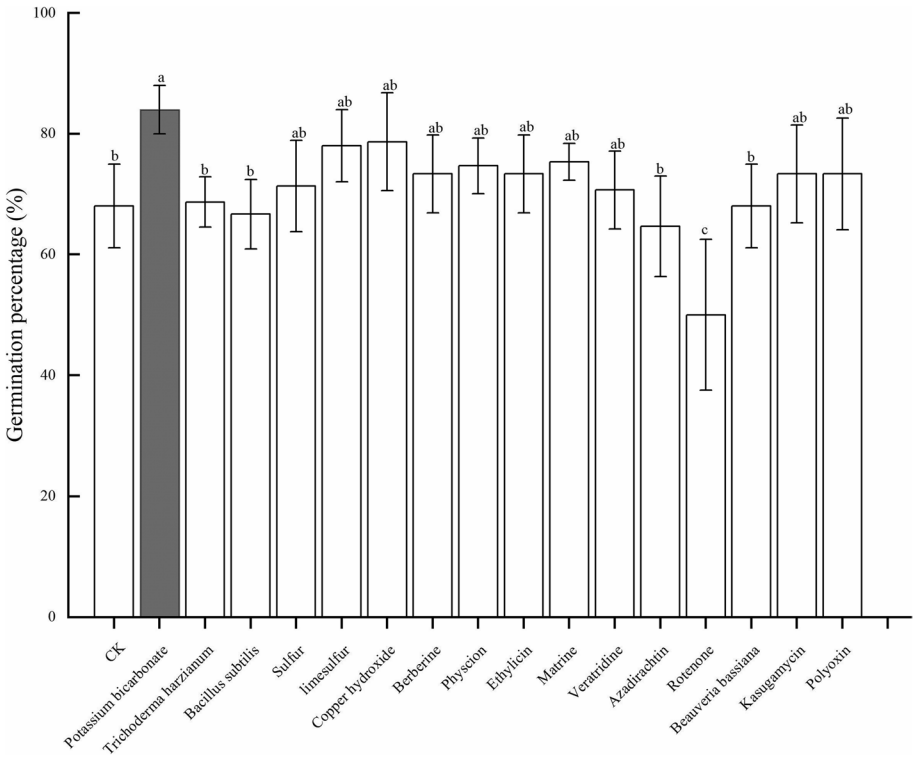

Fig. 6 Seed germination after priming with different types of products

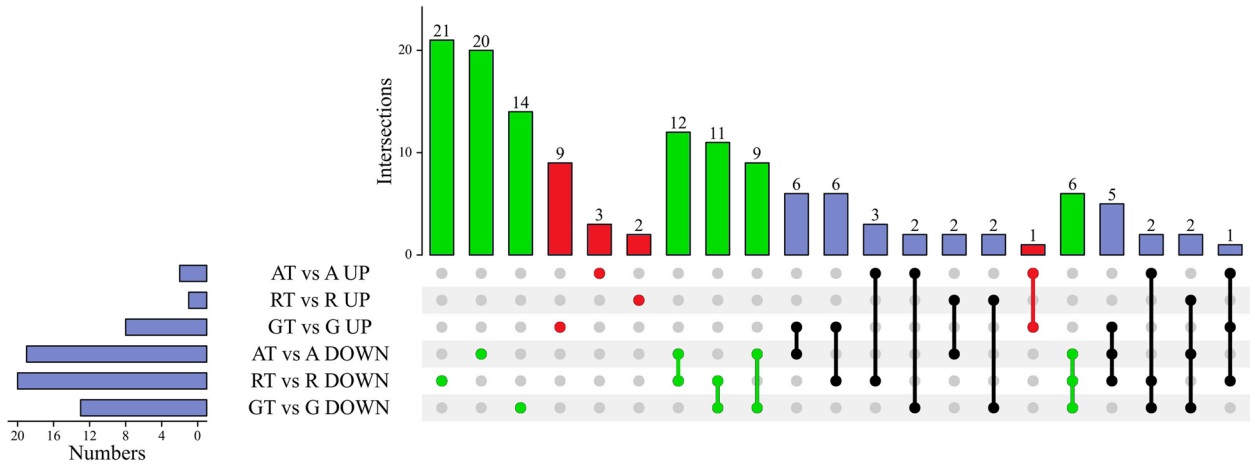

Fig. 7 Antagonistic effect of $KHCO_3$ on fungi in infected sorghum seeds. Before and after $KHCO_3$ treatment (T), the abundance of fungal genera was compared within and between combinations for seeds harvested from Anlong (AT vs A), Renhuai (RT vs R) and Guiyang (G vs GT). The bar chart on the left indicates the number of fungal genera in each comparison. The histogram above indicates the number of fungal genera in the multiple comparisons. Multiple comparisons are indicated by colored lines and connected dots

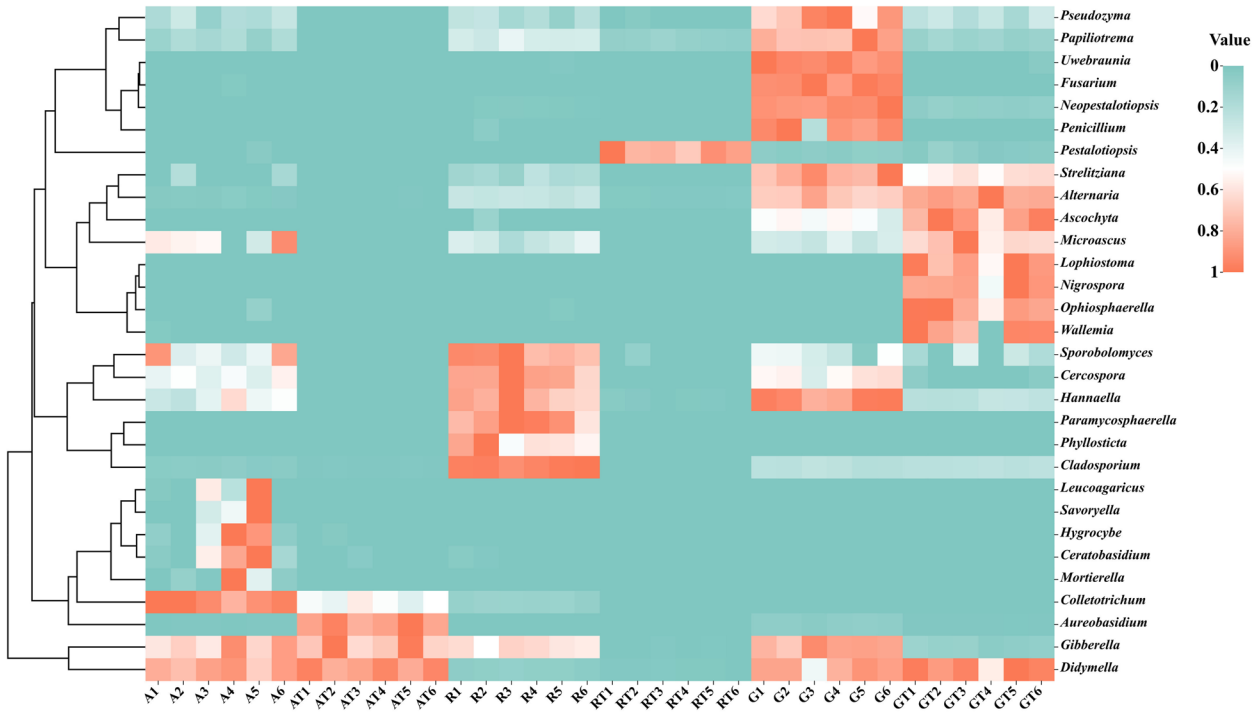

Fig. 8 Role of $KHCO_3$ on fungal diversity of sorghum infected seeds. Before and after $KHCO_3$ treatment (T), the fungal diversity of 6 repetitions of each treatment was compared within and between combinations for seeds harvested from Anlong (AT vs A), Renhuai (RT vs R) and Guiyang (G vs GT). The color of the scale changes from green to red to indicate an increase in the abundance of the genus

the analysis of variance (ANOVA) demonstrated highly significant differences (Fig. S3).

Based on the LC–MS non-targeted metabolomics analysis of sorghum seeds primed with $KHCO_3$ and water for 24 h, a total of 910 metabolites were identified. The most abundant class of metabolites identified was lipids and lipid-like molecules, which accounted for 379 of the total metabolites (Fig. S4). $KHCO_3$ treatment resulted in the upregulation of 647 metabolites and the downregulation of 263, including

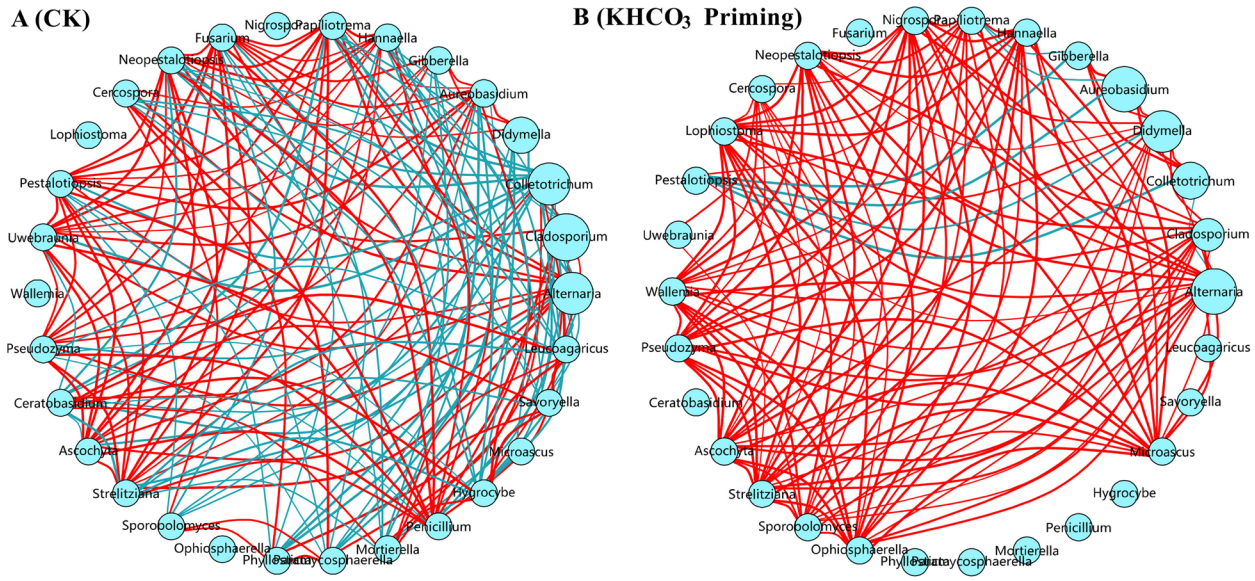

Fig. 9 Comparison of genus networks between (**A**) infected seeds and (**B**) $KHCO_3$-primed seeds in sorghum. The size of the green circles represents the abundance of the bacteria, and the relationships among the genera were linked by blue lines (negative correlation) and red lines (positive correlation)

189 metabolites that were significantly upregulated and 71 that were significantly downregulated (Fig. S5). Priming with $KHCO_3$ induced distinct clustering patterns in the metabolite profiles of germinating sorghum seeds (Fig. 10), indicative of a metabolic divergence between seeds subjected to $KHCO_3$ and water priming. The differentiation between CK0 and T0 was subtle, while the separation between CK0 and T1, as well as between CK1 and T1, was pronounced, suggesting that after 6 h of $KHCO_3$ treatment, the metabolic changes in sorghum seeds were minimal. However, after a 24-h imbibition period, $KHCO_3$ significantly reshaped the metabolic profile of the sorghum seeds (Fig. S6).

Among the cyanoamino acids, levels of L-valine, phenylacetonitrile, and serine were downregulated, in contrast to 2-phenylalanine, γ-glutamyl-p-cyanoalanine, and L-asparagine, which were significantly upregulated. Within the phenylalanine derivatives, caffeoglucaric acid and umbelliferone levels decreased, whereas caffeogl shikmic acid, p-coumaric acid, and corynoline levels increased. The flavonoid biosynthesis pathway generally showed a trend towards downregulation, with significant downregulation observed for naringin, naringenin, and (-) epicatechin. Notably, the phenylalanine intermediate p-coumaroyl-CoA was associated with a significant reduction in naringenin levels, subsequently affecting the levels of other flavonoids. No significant correlation was observed between cyanoamino acids and phenylalanine compounds within the KEGG signaling pathway (Fig. 11, Fig. S7).

Discussion

The microbial community within sorghum seeds has a significant impact on seed quality, severely affecting germination, nutrient absorption, seedling morphogenesis, and disease transmission. Anthracnose is one of the common fungal diseases in crops. Traditionally, the pathogenic fungus causing sorghum anthracnose was considered to be *Colletotrichum graminicola* [*C. graminicola* (Ces.) G. W. Wilson], which grouped all anthracnose fungi occurring on Gramineae plants into a single species. However, Sutton proposed that *C. graminicola* and a separate species, *C. sublineolum*, should be distinguished, with *C. graminicola* primarily infecting maize and *C. sublineolum* parasitizing sorghum [*Sorghum bicolor* (L.) Moench] and other Sorghum species [33]. Before 2009, the name *C. sublineolum* Henn. ex Sacc. & Trotter was frequently used, but it was later recognized that *'sublineolum'* was a misnomer, with *'sublineola'* being the correct variant. At present, the *species* name *C. sublineola* is widely accepted. In this study, *C. sublineola* was also isolated from sorghum seeds and was found to be pathogenic to the seedlings during the germination process of the sorghum seeds.

Seed technologies, such as coating, priming, and physical sterilisation, have been extensively studied for the biological control of seed-borne diseases. Coating seeds with carvacrol and thymol has been shown to significantly suppress anthracnose, caused by *C. gloeosporioides*, in mango and papaya [34]. Similarly, priming seeds with *Bacillus subtilis* has been found to substantially

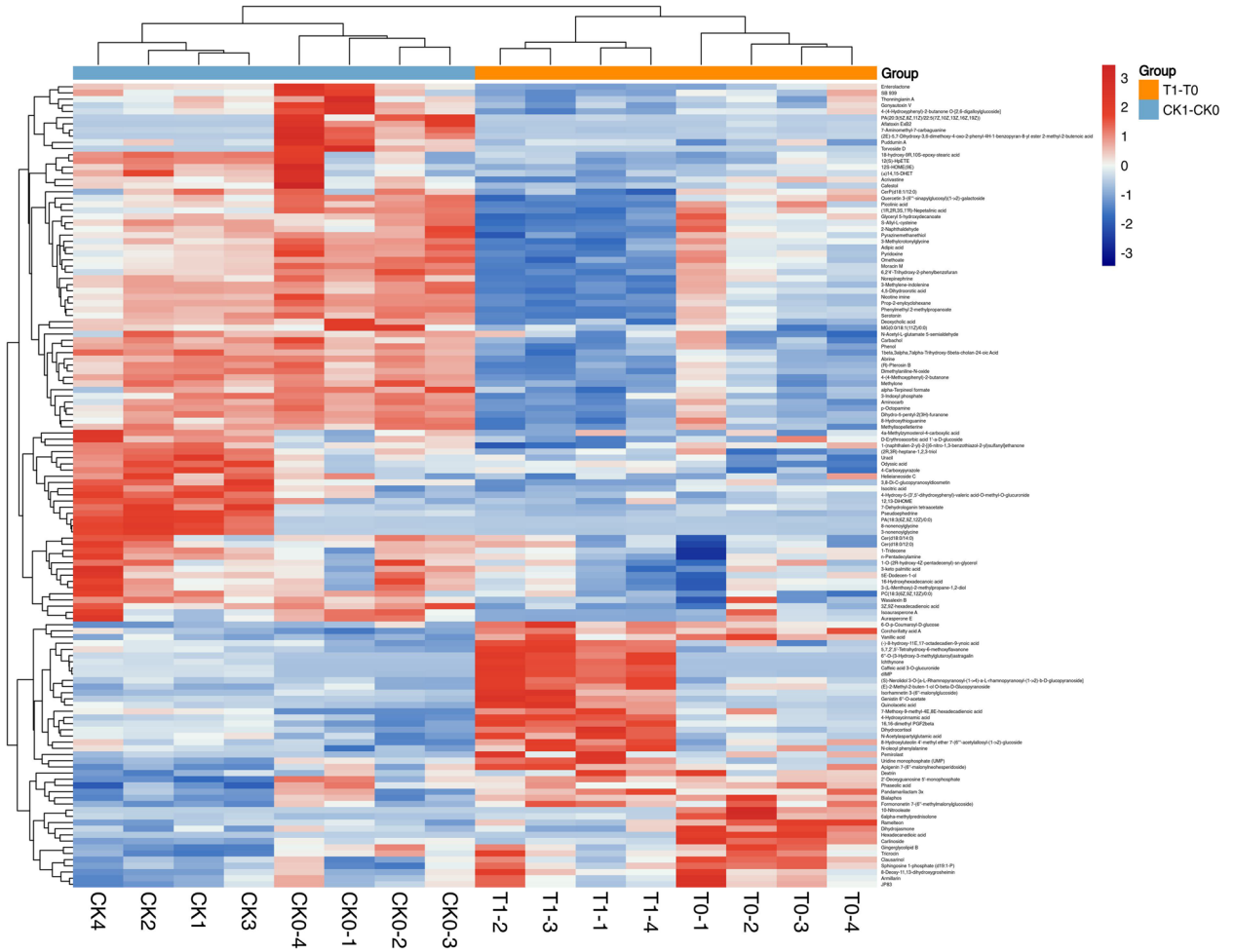

Fig. 10 Changes in metabolite clustering grouping after $KHCO_3$ priming

reduce the incidence of seed-borne anthracnose in peppers, caused by C. capsici [23]. However, microbicides typically require refrigeration to preserve cell viability, which escalates the cost of disease management and limits their applicability [35]. Seed irradiation with UV-C has also been demonstrated to significantly suppress seed-borne anthracnose in lupin, caused by *C. acutatum* [27]. Nonetheless, physical methods are often intricate and necessitate the use of complex, costly equipment, rendering them challenging to implement in practical agricultural settings [36]. In light of the challenges associated with conventional seed treatments, there is an imperative need for innovative seed technologies that are environmentally sustainable and capable of maintaining effective fungicidal levels. Seed priming with highly targeted biocompatible chemicals presents a viable alternative, as it facilitates comprehensive control of seed-borne pathogens both on the seed surface and internally, at a reduced cost and dosage. For example, priming with salicylic acid has proven effective in diminishing the incidence of wilt in wheat, caused by *Fusarium graminearum*, and enhancing resistance [37]. The application of nanoparticle-mediated priming to chickpea seeds has been shown to effectively suppress Fusarium wilt, a disease caused by *Fusarium oxysporu*m [38]. Seed priming with cholic acid glycine conjugates has significantly mitigated the incidence of bacterial blight and sheath blight diseases, which are respectively caused by *Xanthomonas oryzae pv. oryzae* and *Rhizoctonia solani* [39]. In sorghum, seed priming with *Trichoderma harzianum* has been effective in controlling anthracnose disease caused by *C. sublineola* [22, 26]. In this study, $KHCO_3$ priming was identified as an efficacious seed treatment for managing seed-borne anthracnose caused by *C. sublineola*, *C. gloeosporioides*, and *C. fructicola*. Beyond the disinfection of seed-borne pathogens, bio-priming technology has also been observed to enhance seed germination in certain crops. For instance, chitosan priming has been

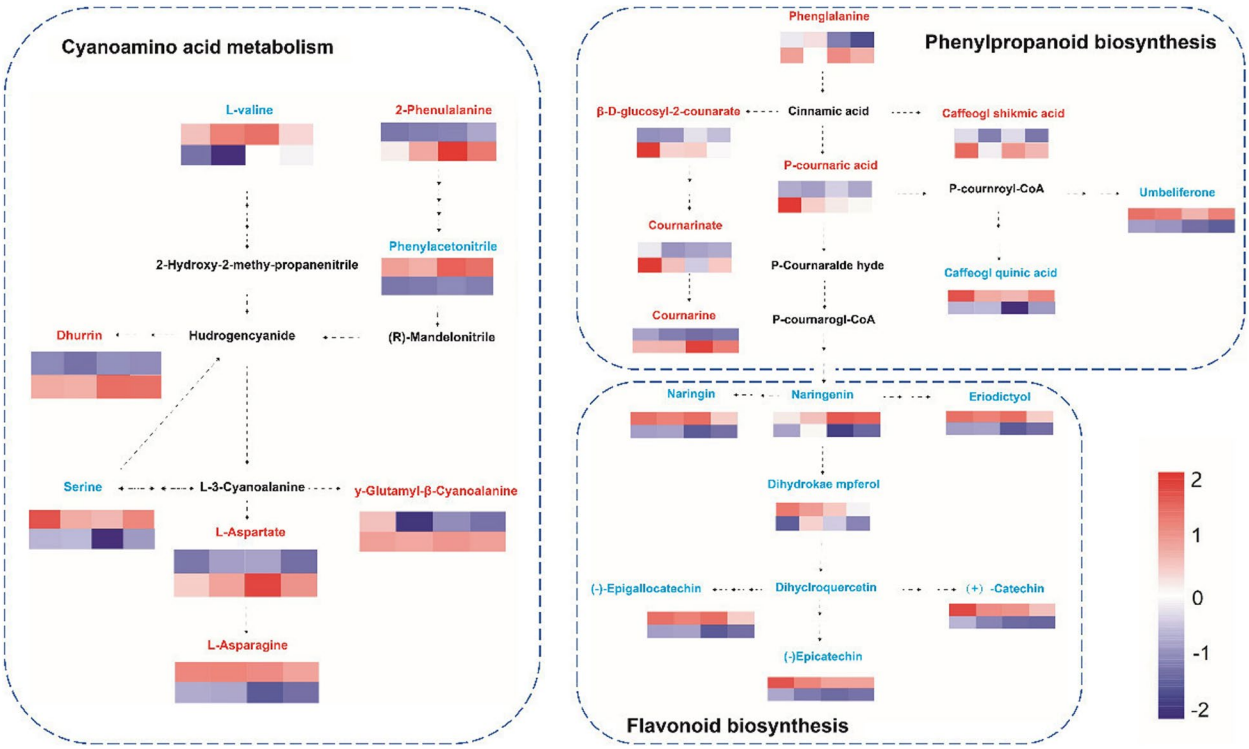

Fig. 11 $KHCO_3$ priming metabolic pathways of major differential metabolites in sorghum seeds. The heat map in the grid shows the water priming imbibition for 24 h (CK1), and the bottom shows ($KHCO_3$ priming imbibition for 24 h) T1. Blue fonts indicate down-regulation, red fonts indicate up-regulation, and black represents no significant change

shown to suppress powdery mildew and improve germination in cucumber [40]. The use of plant gum biopolymers in seed priming not only controls downy mildew but also enhances the germination of pearl millet seeds [41]. Ascorbic acid priming has been demonstrated to control Fusarium wilts and improve seed germination in tomatoes [42]. Similarly, this study found that $KHCO_3$ priming disinfected seed-borne pathogens and increased germination rates in sorghum.

$KHCO_3$ is a globally recognized safe compound widely used as an antifungal agent in fruits and flowers. It has demonstrated effectiveness against various fungal diseases, such as white rust in chrysanthemums [43], grey mould in kiwifruit [32], anthracnose in citrus [44], and strawberry [45]. However, its application as an antifungal agent for controlling seed-borne pathogens in crops has been limited. Recent research has reported that treating seeds with $KHCO_3$ can effectively control seedling damping-off in cotton [46]. The PCoA analysis also revealed that $KHCO_3$ priming significantly affected the composition and diversity of the seed-borne fungal community. Cluster analysis showed that in newly harvested seeds from the three regions, the abundance of *Pseudozyma*, *Papiliotrema*, *Sporobolomyces*, *Cercospora*, *Hannaella*, and *Colletobasidium* was significantly reduced in the $KHCO_3$-treated seeds. FUNGuild and network structure analysis indicated a significant reduction in negative correlations following $KHCO_3$ priming, with Hygrocybe, Penicillium, Mortierella, *Paramycosphaerella*, *Phyllosticta*, *Ceratobasidium*, and *Fusarium* becoming independent of other genera. Furthermore, the abundance of fungi in 15 genera, including *Colletotrichum*, *Cercospora*, and *Stagonospora*, decreased in $KHCO_3$-treated seeds from the three regions. A few genera, such as *Aureobasidium*, showed an increase in fungal abundance. In summary, this study suggests that $KHCO_3$ can suppress *Colletotrichum* species and alter the fungal community structure and abundance within sorghum seeds.

This study discovered that $KHCO_3$ priming leads to the upregulation of the metabolite dhurrin in the cyanoamino acid metabolism of sorghum plants. Dhurrin, a defense compound, serves a dual purpose. On one hand, it decomposes into hydrogen cyanide, enhancing resistance against biotic stress [47]. On the other hand, the decomposition of dhurrin releases an aldoxime intermediate, which exhibits antifungal effects [48]. Therefore, it is hypothesized that $KHCO_3$ triggers an increase in dhurrin metabolite levels, thereby enhancing the antifungal ability by modulating the cyanoamino acid metabolism. During seed germination and seedling growth, significant

amounts of flavonoids are produced. The flavonoid biosynthetic pathway exhibits differential expression trends in genes involved in seed imbibition and germination. Flavonoids are synthesized through the downstream flavonoid pathway, which is part of the phenylpropanoid biosynthesis. Previous studies have demonstrated that a decrease in naringenin, an upstream component of the flavonoid synthesis pathway, can upregulate downstream metabolites such as Hesperetin7-0-glucoside, Rhoifoliin, Quercetin 3-sulfonate, and Baimaside, benefiting seed germination and seedling growth [49]. This aligns with the findings of this study, where P-cournarogl-CoA in phenylalanine led to a significant decrease in Naringenin within the flavonoids. It suggests that $KHCO_3$ priming improves sorghum seed germination by influencing the flavonoid pathway through the phenylalanine pathway. Additionally, flavonoids also play a crucial role in plant defense against both biotic and abiotic stresses. The differentially expressed genes (DEGs) and metabolites involved in flavonoid biosynthesis contribute significantly to seed germination and seedling growth. This study, for the first time, highlights the dual effect of $KHCO_3$ on sorghum seeds, inhibiting seed-borne diseases while promoting germination. Not only $KHCO_3$, but also other bicarbonates can prevent against fungal diseases. For example, the salt ion of sodium bicarbonate prevents and controls root rot pathogens in beans [30]. Ammonium bicarbonate and sodium bicarbonate are effective in controlling anthracnose in bananas [50]. Thus, bicarbonate ions may be responsible for the inhibition of seed-borne anthracnose by $KHCO_3$, the informative evidence for which requires follow-up studies.

Our experiments showed that $KHCO_3$ inhibited the *Colletotrichum* abundance of harvested seeds in all three regions. In addition, germination and seedling emergence were significantly higher in $KHCO_3$ primed seeds compared to unprimed seeds. Fungal abundance of 15 genera, such as *Colletotrichum*, *Cercospora* and *Stagonospora,* were decreased in infected seeds from all three regions following $KHCO_3$ priming, while only a few genera, such as *Aureobasidium*, were significantly increased. *Aureobasidium* has been reported to be a biological agent with antibacterial activity against bacteria and fungi [51]. To sum up, we speculatived that $KHCO_3$ will change the structure of fungal communities in sorghum seeds and promote seed germination.

Conclusions

Seed-borne diseases have seriously affected the sustainability of sorghum cultivation in China as the demand for organic products in the winemaking industry has limited the use of chemical fungicides. The study initially involved collecting sorghum seeds from three major sorghum-growing regions in Guizhou Province. We then employed traditional tissue separation and high-throughput sequencing methods to analyse the diversity of fungi carried by the sorghum seeds, identifying *Colletotrichum*, *Fusarium*, *Cladosporium*, and *Alternaria* as the predominant fungal genera associated with sorghum seeds. Subsequently, we conducted pathogenicity tests on the isolated fungi and found that strains GD202206, GD202219, and GD202242 were pathogenic. Through morphological and the construction of a multi-gene phylogenetic tree, they were identified as *C. sublineola*. We then selected 16 fungicides in China's organic directory for seed priming experiments with sorghum seeds. The results indicated that priming with $KHCO_3$ significantly enhanced germination of the sorghum seeds, with both indoor and outdoor emergence rates notably higher than those of the control (CK).Analysis of the fungal changes before and after $KHCO_3$ priming revealed a significant reduction in the abundance of the *Colletotrichum* genus. Additionally, $KHCO_3$ altered the community structure of fungi within the sorghum seeds, reducing population richness. Inter-generic relationships were rebalanced, with antagonism decreased and synergy increased following $KHCO_3$ treatment. Non-target metabolomic analysis indicated that $KHCO_3$ enhances sorghum seed germination via the phenylalanine and flavonoid pathways and exhibits antifungal properties through the cyanoamino acid metabolic pathway. In summary, this study revealed that $KHCO_3$ had a dual effect on sorghum seeds, not only inhibiting seed-borne pathogens but also promoting seedling emergence.

Materials and methods

Materials

Hongyingzi is the most widely planted sorghum variety in China and is also the primary sorghum variety promoted in Guizhou Province. It is the designated raw material variety for the sauce-flavored liquor "Moutai" from Guizhou. The sorghum seeds of the Hongyingzi variety were supplied by Hongyingzi Agricultural Technology Co Ltd, a subsidiary of the Maotai Group of companies, and were planted in 3 locations of Renhuai (R), Anlong (A) and Guiyang (G) City in guizhou province. All seeds were harvested after filling and mechanically dried at 40 °C for 30 h. After threshing, the seeds were stored at -10 °C.

Seed priming

Sixteen products were selected for subsequent experiments, based on a literature review, as they have demonstrated efficacy in other studies. All of these products are officially approved environmentally friendly antifungicides suitable for commercial trade, as shown in

Table S1. Each of the 16 products was individually dissolved in ddH_2O aqueous solutions, and sorghum seeds were poured into these solutions to be primed at 18 °C for 12 h. Subsequently, the primed seeds are re-dried to a moisture content of 7% ~ 11% at 38 °C and 45% relative humidity. Finally, the re-dried seeds are stored at -10 °C.

Germination and emergence test

Germination experiments were performed on filter paper beds with 3 replicates of 100 seeds each. Seeds were cultured in an artificial climate chamber (RTOP-400Y, Topun Technology Co. of Zhejiang, China) at 25 °C, 75% humidity and a 12-h photoperiod. Germination percentages were recorded on day after inhibition of 7, and the criterion for germination completed was that the observed radicle length was approximately equal to the seed length. Germination percentages = (number of germinated seed/the total number of seeds) × 100%.

Seedling emergence experiments were carried out in 40 cm × 40 cm pots, with three replications of 50 seeds each. Seeds or seedling were grown under natural conditions with a temperature range 15 to 25℃, and the soil humidity greater than 70%. The standard for the emergence is two cotyledons emerging from the soil. The seedling emergence rate was calculated 20 days after sowing, and the seedling emergence rate = (the number of emerged seedlings/the total number of seeds) × 100%.

Fungal isolation

Seeds were observed one by one with an NSZ818 stereomicroscope (Nexcope, Ningbo, China). 100 infected seeds were randomly selected according to their mycelium, galls, sclerotia, nematode galls and colonies. The infected seeds were disinfected with 5% sodium hypochlorite solution for 5 min, rinsed three times with sterile water, disinfected with 75% alcohol for 30 s and rinsed three times with sterile water. The surface disinfected seeds were placed on PDA plate and incubated at 25 °C for 5 days. Each colony around the seeds was transferred to a new PDA plate by scratching, and the isolation and purification were repeated 3 to 5 times using the hyphal tipping method until a single colony was purified. The strains were stored in 25% glycerol at 4 °C. The fungal cultures were transferred onto PDA medium and incubated at 25 °C for 7 days before use.

Pathogenicity tests

Seed Inoculation: Select intact sorghum seeds at random and disinfect them. After drying the residual moisture on the seed surface with sterile filter paper, immerse the seeds in a spore suspension of the test fungal strain for 2 h (concentration of 1×10^6 spores/mL^{-1}). Subsequently, sow the seeds in germination boxes filled with sterile soil, with 10 seeds per box and four replicates. After inoculating the seeds with the pathogen, incubate them in a lighted incubator at 25 °C for 7 days. Daily observations were made to check for disease symptoms, including the appearance of lesions on the seeds and wilting or necrosis on the stems. Infected areas were then examined under a microscope to observe the consistency of the morphology of the lesions.

Leaf Inoculation: Choose healthy seeds and sow them in soil that has been sterilised. Inoculation was carried out when the sorghum seedlings reached the four-leaf stage with a central shoot, using a spray method. (concentration of 1×10^6 spores/ mL^{-1}). Cultivate in a lighted incubator at 25 °C for 7 days and monitor for lesion formation and necrosis. Infected areas were then examined under a microscope to observe the consistency of the morphology of the lesions.

Morphological and molecular characterization

Total genomic DNA was extracted from the colony of the three pathogenic isolates using a CWBIOTECH Plant Genomic DNA Kit (Beijing, China) following the manufacturer' sprotocol. The primers used for PCR amplification and the amplification conditions are listed in Table S2. The PCR products were electrophoresed for detection; if a clear, bright, and single band of the expected size was present, the amplified products were sent to Sangon Bioengineering (Shanghai) Co., Ltd. for sequencing. The obtained DNA sequences were assembled and corrected using ContigExpress software. The sequence was then subjected to homology comparison analysis using the BLAST program in NCBI, gathering information on related strains from the literature. High similarity strain gene sequences were downloaded from GenBank, with the accession information listed in Table 1. Multiple sequence comparisons were performed using BioEdit V7.0.5 software. Through the CIPRES website, a phylogenetic tree was constructed using the Maximum Likelihood (ML) method to determine the genus and species classification of the pathogen.

High-throughput sequencing technology is utilized to analyze the diversity of fungi carried by sorghum seeds

Samples of sorghum seed were rapidly frozen and stored at -80 °C. Total DNA was isolated from the samples using a DNeasyPowerSoil kit (Qiagen, Hilden, Germany). DNA concentration and integrity were detected by a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively. The ITS1 variable region was amplified using a universal primer pairs (ITS1F: 5′-CTT GGTCATTTAGAGGAAGTA-3′; ITS2: 5′- GCTGCG TTCTTCATCGATGC -3′). Reverse primer contained

Table 1 Information of *Colletotrichum* spp. used in this study for phylogenetic analyses

Species	Strains	GenBank accession number				
		ITS	***GAPDH***	***CHS-1***	***ACT***	***TUB2***
C. boninense	CNU220003	ON863699	ON863702	-		ON863705.1
D. *boninense*	SZ-1	OP954625	OP966685	-	OP966690	OR075970
C. boninense	YB15	-	-	-		
C. boninense	ACCC 35480	OR240823	OR251067	-	OR251088	OR251095
C. cliviicola	Co-ZX-2	-	-	-	-	-
C. graminicola	MANE53	-	-	-	-	-
C. cattleyicola	CBS 170.49	MG600758	MG600819	MG600866	MG600963	MG601025
C. clivicola	CBS 170.49	MG600733	MG600795	MG600850	MG600939	MG601000
C. clivicola	CORCX9	HM585398	HM585381	HM582025	HM581986	HM585423
C. cliviicola	ZG-DA-2	MK881738	MK881739	MK881740	MK881741	MK881742
C. cliviicola	ST120	MH291214	MH291258	MH291236	MH292789	MH458027
C. navitas	CBS 125086	JQ005769	-	JQ005790	JQ005832	JQ005853
C. plurivorum	KJF0002	OP926032	OQ082300	-	-	OQ082302.1
C. plurivorum	LFN0079	JQ926743	MK862217	-	-	MK814942
C. plurivorum	URM 8640	OQ130748	-	-	OQ144656	OQ144657
Colletotrichum sp.	HSG826-P5.1	OQ263015	OQ282973	OR004793	OQ282966	OQ282980
C. tofieldiae	LYZ0664	PP421934	PP426206.1		PP426200	PP426197
C. sublineola	CQ-ZX-1	MK881648	MK881665	MK881682	MK881699	MK881716
C. sublineola	CQ-ZX-1	MK881661	MK881678	MK881695	MK881712	MK881729
C. sublineola	YB-CN-1	MK881655	MK881672	MK881689	MK881706	MK881723
C. sublineola	YB-NX-1	MK881653	MK881670	MK881687	MK881704	MK881721
C. sublineola	YN-YJ-1	MK881664	MK881681	MK881698	MK881715	MK881732
C. sublineola	YB-JA-1	MK881654	MK881671	MK881688	MK881705	MK881722
C. sublineola	ZG-DA-1	MK881651	MK881668	MK881685	MK881702	MK881719
C. sublineola	ZG-FS-1	MK881652	MK881669	MK881686	MK881703	MK881720
C. sublineola	SD-WF-1	MK881663	MK881680	MK881697	MK881714	MK881731
C. sublineola	CQ-YC-1	MK881648	MK881665	MK881682	MK881699	MK881716
C. sublineola	**GD202206**	**ON680862**	**PP763563**	**PP763564**	**OP056614**	**PP763566**
C. sublineola	**GD202219**	**PP403902**	**PP500986**	**PP500990**	**PP500981**	**PP500984**
C. sublineola	**GD202242**	**PP403903**	**PP500985**	**PP500989**	**PP500982**	**PP500983**
Monilochaetes infuscans	CBS869.96	JQ005780	JX546612	JQ005801	JQ005843	JQ005864

a sample barcode and both primers were ligated using an Illumina sequencing adapters. Amplicon quality was visualised by gel electrophoresis. PCR products were purified using AgencourtAMPure XP beads (Beckman Coulter Co., USA) and quantified using the Qubit dsDNA detection kit. Concentrations were then adjusted for sequencing. Sequencing was performed on an Illumina Miseq with two paired read cycles of 300 bases each (Illumina Inc., San Diego, CA).

Paired reads were pre-processed using Trimmomatic software [52] to detect and cut off ambiguous bases (N). It also used a sliding window trimming method to cut off low quality sequences with an average quality score below 20. After trimming, paired reads were assembled using FLASH software [53]. The parameters for assembly were: Minimum overlap of 10 bp, maximum overlap of 200 bp and a maximum mismatch rate of 20%. Sequences were further de-mismatched as follows: reads with ambiguous, homologous sequences or below 200 bp were discarded. Using QIIME software (version 1.8.0) [54]. Then, 75% of the reads with bases above Q20 were retained. Reads with chimeras were then detected and removed using VSEARCH [55]. Primer sequences were removed and clean reads were clustered using VSEARCH software to produce operational taxonomic units (OTUs) with 97% similarity [55]. Microbial diversity in sorghum seed samples was estimated using alpha diversity. Unifrac distance matrices performed by QIIME software were used for unweighted Unifrac Principal Coordinate Analysis (PCoA) and phylogenetic tree construction.

LC–MS metabolomics detection

Undried seeds after 6 h (T0) and seeds imbibed for 24 h (T1) were induced by $KHCO_3$. Controls were seeds that were not dried after water priming for 6 h (CK0) and seeds that were imbibed for 24 h (CK1). Place the above materials on absorbent paper in the clean workbench. After the surface moisture was absorbed, they were quickly frozen in liquid nitrogen until complete metabolic inactivation. Each group of samples was repeated 4 times.

Take 60 mg sample and place it in a 1.5 mL centrifuge tube, add 600 µL methanol–water (V:V = 7:3, containing L-2-chlorophenylalanine, 4 µg/mL), and store it in a -40 °C refrigerator Leave it for 2 min to pre-cool, add two small steel beads, grind in a grinder at 60 Hz for 2 min, ultrasonic extraction in an ice water bath for 30 min, then let it stand at -40 °C overnight, centrifuge at 13,000 rpm for 10 min at 4 °C, and draw 150 µL of the supernatant. The clear liquid was filtered using a 0.22 µm organic phase pinhole filter, transferred to an LC injection vial, and stored at -80 °C until LC–MS analysis. Quality control samples (QC) were prepared by mixing equal volumes of extracts from all samples. LC–MS metabolic analysis uses an LC–MS system composed of ACQUITY UPLC I-Class plus ultra-high performance liquid phase tandem QE high-resolution mass spectrometer. The chromatographic column used is ACQUITY UPLC HSS T3 (100 mm × 2.1 mm, 1.8 µm), the mobile phase is ultrapure water (containing 0.1% formic acid), the organic phase is acetonitrile, the flow rate is 0.35 mL/min, and the column temperature is 45 °C. The injection volume is 5 µL. The sample mass spectrum peaks were collected using positive and negative ion scanning modes.

Data analysis

Alpha diversity index analysis of microbial diversity in sorghum seeds using QIIME software. FUNGuild analysis of seed-borne fungal communities by FUNGuild (Fungi Functional Guild). Data with a correlation greater than 0.1 and a *P* value less than 0.05 were screened to construct a correlation network, and a fungal correlation network diagram was drawn based on Python.Metabolomics data were analyzed using MetaboAnalyst 5.0 (http://www.metaboanalyst.ca/). Combine the VIP value of the OPLS-DA model with the difference fold to screen differential metabolites and the screening criteria were $|\log2FC| > 1$, $VIP > 1$. The KEGG database was used to functionally annotate differential metabolites, and KEGG pathway enrichment analysis was performed based on the annotation results. Statistical analyses were performed by IBM SPSS Statistics 26.0 software, and figures were plotted by Orign2018a. The differential expression heatmaps were plotted using Chiplot Oline (https://www.chiplot.online/), and PCA analysis was performed by using R language.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03824-9.

Supplementary Material 1.
Supplementary Material 2.
Supplementary Material 3.
Supplementary Material 4.
Supplementary Material 5.
Supplementary Material 6.
Supplementary Material 7.
Supplementary Material 8.
Supplementary Material 9.

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Authors' contributions

Shuai Dong and Keqin Peng: Formal analysis, Writing-original draft, Investigation, Revision. Qi yuan Liu: Investigation, Writing-review. Ming Zhang: Sampling, Investigation, Writing-draft preparation. Yan Luo: Investigation. Zhen hua Li: Supervision, Resources, Writing-original draft, Revision.

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Data availability

The datasets generated during the current study, which include the sequences of the three isolates identified as GD202206, GD202219, and GD202242, are available in the GenBank repository under the following accession numbers: ITS: ON680862, PP403902, PP403903; GAPDH: PP763563, PP500986, PP500985; CHS-1: PP763564, PP500990, PP500989; ACT: OP056614, PP500981, PP500982;TUB2: PP763566, PP500984, PP500983.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]College of Agriculture/Institute of Rice Industry Technology Research, Key Laboratory of Plant Resource, Guizhou University, Guiyang 550025, China. [2]Key Laboratory of Molecular Breeding for Grain and Oil Crops in Guizhou Province, Guiyang 550025, China. [3]Qiannan Academy of Agricultural Sciences, Duyun 558000, China. [4]Key Laboratory of Functional Agriculture of Guizhou Provincial Higher Education Institutions, Guiyang 550025, China.

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