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ORIGINAL ARTICLE

Deciphering the antagonistic effect of *Streptomyces* spp. and host-plant resistance induction against charcoal rot of sorghum

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Abstract

Main conclusion **The findings of this study suggest that the selected five strains of Streptomyces spp. could be used for biological control of charcoal rot disease in sorghum.**

Abstract Two strains each of *Streptomyces albus* (CAI-17 and KAI-27) and *Streptomyces griseus* (KAI-26 and MMA-32) and one strain of *Streptomyces cavourensis* (SAI-13) previously reported to have plant growth-promotion activity in chickpea, rice and sorghum were evaluated for their antagonistic potential against *Macrophomina phaseolina*, which causes charcoal rot in sorghum. The antagonistic potential of these strains against *M. phaseolina* was assessed through dual culture assay, metabolite production assay, blotter paper assay in greenhouse and field disease screens. In both dual culture and metabolite production assays, the selected strains significantly inhibited the growth of *M. phaseolina* (63–74%). In the blotter paper assay, all the five strains of *Streptomyces* spp. inhibited the pathogen (80–90%). When these five strains were tested for their antagonistic potential under the greenhouse (two times) and field (two seasons) conditions by toothpick method of inoculation, significant differences were observed for charcoal rot severity. Principal component analysis capturing 91.3% phenotypic variations, revealed that the shoot samples treated with both *Streptomyces* and the pathogen exhibited significantly enhanced antioxidant parameters including superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase, glutathione reductase, phenylalanine ammonia-lyase, polyphenol oxidase, and total phenolic contents when compared to shoot samples treated with only *M. phaseolina*. Scanning electron microscope analysis revealed that the phloem and xylem tissues of the *Streptomyces* treated stem samples were intact compared to that of pathogen inoculated plants. This study indicated that the selected strains of *Streptomyces* spp. have the potential for biological control of charcoal rot disease in sorghum.

Keywords *Streptomyces* spp. · Biological control · Charcoal rot · Sorghum · *Macrophomina phaseolina*

Introduction

Macrophomina phaseolina (Tassi) Goid., is a devastating plant pathogen that causes charcoal rot disease in more than 500 agriculturally important crops including common bean, soybean, chickpea, sunflower, maize, geranium, tomato and sorghum (Kaur et al. 2012; Jordaan et al. 2019). Charcoal rot infection is distinct from other diseases such as seedling blight, stem rot, collar rot and root rot by its characteristic charcoal-like appearance in the infected parts of the plant (Kaur et al. 2012). High temperature and low soil moisture are the important factors predisposing sorghum plants to infection by *M. phaseolina.* As irrigation is withheld at 50% flowering as per the screening protocol, plants start experiencing moisture stress after 10–15 days which facilitates *M.*

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phaseolina infection. *M. phaseolina* retains its pathogenicity for a period of up to 3 years in the soil from the host plant decomposition (Kaur et al. 2012). Sorghum (*Sorghum bicolor* L. Moench) is one of the most affected crops by *M. phaseolina* after soybean and can cause up to 100% yield loss under favourable conditions (Patil and Kamble 2011). Sorghum serves as a staple food crop for more than 500 million populations in Asia and sub-Saharan Africa.

Worldwide, many attempts are being made by researchers to control this disease, which affects large areas of post-rainy (Rabi) sorghum production fields (3–4 m ha) and causes a significant loss in yield (Ghosh et al. 2018). The biggest problem in managing this disease is the lack of a high level of resistance in the post-rainy adapted sorghum cultivars. However, there are some novel crop management methods in practice to overcome the charcoal rot in sorghum. For instance, application of fungicides such as carbendazim and thiram (Manjeet and Umesh 2013); gamma-ray irradiation for development of disease tolerant varieties (Ashok et al. 2018); development of disease-resistant sorghum varieties along with their evaluation in multiple locations (Das et al. 2018); application of plant growth-promoting microbes such as *Trichoderma* sp., *Pseudomonas* sp., *Bacillus* sp., *Klebsiella* sp., *Amycolatopsis* sp., *Acinetobacter* sp. and *Enterobacter* sp. (Khaledi and Taheri 2016; Torres et al. 2016; Dey et al. 2019; Gopalakrishnan et al. 2019). Even though, various approaches to fight against *M. phaseolina* are available still there is a gap to be filled to enhance the tolerance levels in sorghum against this pathogen. *M. phaseolina* induces host cell wall degrading enzymes in sorghum which promotes the susceptibility of infection (Bandara et al. 2018). This shows the importance of understanding sorghum-*M. phaseolina* interactions further and identifying more potent biocontrol strategies that could be combined with a moderate level of tolerance in the host to manage charcoal rot.

Streptomyces is the largest genus of actinobacteria representing 50% of the total population of soil actinobacteria (Parte et al. 2020). *Streptomyces* is a filamentous bacterium, having high G + C content, representing the family *Streptomycetaceae* and order Actinomycetales (Gopalakrishnan et al. 2020a). It is well known for its prolific production of bioactive compounds. About 75% of the reported antibiotics were identified from *Streptomyces* (Olanrewaju and Babalola 2019). The filaments of *Streptomyces* help to absorb the maximum amount of the nutrients from the soil and supports colonization on substrates especially on the plant roots. Due to this reason, the population of *Streptomyces* is generally highly recorded in strain isolation, metagenomics, genome mining and sequencing (Newitt et al. 2019). Apart from their plant growth promotion traits, *Streptomyces* are also known for their biocontrol potential against many phytopathogens. For instance, *Streptomyces* sp. RP1A-12 has been reported to reduce stem rot (caused by *Sclerotium rolfsii*) disease incidence by 50% in groundnut (Jacob et al. 2018); *Streptomyces* sp. UPMRS4 reduced the disease severity of *Pyricularia oryzae* in rice (Awla et al. 2017); *Streptomyces* sp. AC-19 inhibited *Fusarium oxysporum* f. sp. *ciceri* in chickpea (Anusha et al. 2019); and *Streptomyces* spp. inhibited the crown rot and root rot causing *Fusarium* spp. (Winter et al. 2019).

Earlier, we reported five strains of *Streptomyces* spp., CAI-17, KAI-26, KAI-27, MMA-32 and SAI-13, identified based on the taxonomic assignment of 16S rRNA sequences against the 16 s rRNA database available then, to have plant growth-promotion potential in chickpea, rice and sorghum under field conditions (Gopalakrishnan et al. 2011a, b, 2012, 2013, 2015a, b). These strains were re-sequenced by whole genome sequencing in the year 2020 and identified up to the species level as *S. albus* CAI-17, *S. griseus* KAI-26, *S. albus* KAI-27, *S. griseus* MMA-32 (Gopalakrishnan et al. 2020b) and *S. cavourensis* SAI-13 (Sreevidya et al. 2016). The main objective of the present study was to screen the five strains of these *Streptomyces* spp. for their antagonistic potential against charcoal rot of sorghum by dual culture assay, metabolite production assay, blotter paper assay and in greenhouse and field screens.

Materials and methods

Streptomyces spp. used in this study

Five strains of *Streptomyces* spp., such as *S. albus* CAI-17 (WGS GenBank accession number: JAANNQ000000000), *S. griseus* KAI-26 (WGS GenBank accession number: JAANNR000000000), *S. albus* KAI-27 (WGS GenBank accession number: JAANNU000000000), *S. griseus* MMA-32 (WGS GenBank accession number: JAANNS000000000) and *S. cavourensis* SAI-13 (GenBank accession number: KM220609) previously reported to have a capacity for plant growth-promotion in sorghum (Gopalakrishnan et al. 2011a, 2020b; Sreevidya et al. 2016) were selected for the present study.

In vitro dual culture assay

The selected five strains of *Streptomyces* spp. were tested for their antagonistic activity against *M. phaseolina* [isolated from infected sorghum plants collected from the disease nursery at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India] by dual culture assay as described earlier (Gopalakrishnan et al. 2011a). In brief, a disk of 6 mm diameter of *M. phaseolina* was placed on one edge of the starch casein agar plate (1 cm from the corner) and the selected *Streptomyces* strain was streaked on the other edge of the plate (1 cm

from the corner), followed by incubation at 28 ± 2 °C for 120 h or till the pathogen covered the entire plate in control. Inhibition of fungal mycelium (halo zone) around the *Streptomyces* colony was scored positive and the inhibition zone was measured.

In vitro metabolite production assay

For this, the five *Streptomyces* strains were grown on starch casein broth (SCB) at 28 ± 2 °C for 7 days. At the end of incubation, the cell-free extract was collected, by centrifuging the culture at 10,000 × *g* for 20 min, and their metabolites extracted by the solvent partitioning method (Westley et al. 1979) using ethyl acetate. The resultant organic and aqueous fractions were tested against charcoal rot pathogen, *M. phaseolina*, by modified poisoned food technique (Alekhya et al. 2016). In brief, potato dextrose agar (PDA) plates with 10% fractions were prepared. A fungal disc with 4 mm diameter of *M. phaseolina* was bored and placed at the centre of the plate. After 5 days of incubation at 28 ± 2 °C, the fungal growth was measured and compared with control plates, where no fractions of organic or aqueous extracts were added.

In vivo blotter paper assay

The selected five strains of *Streptomyces* spp. were evaluated for their biocontrol potential against charcoal rot on sorghum line 296B (susceptible to charcoal rot) by blotter paper assay as described earlier (Gopalakrishnan et al. 2019). In brief, 2-week-old seedlings of 296B were dipped in the inoculum of *M. phaseolina* (grown separately in potato dextrose broth) at 28 ± 2 °C for 30 min followed by dipping in selected *Streptomyces* strain (grown separately in starch casein broth) and placed side by side on a blotter paper (45 × 25 cm) so that only the roots were covered. Positive and negative controls were included by inoculating the plants only with pathogen (*M. phaseolina*) and sterile water, respectively. Twenty plants per replicate with three replications were used for each treatment. The blotter paper was kept moist with sterilized water all the time and incubated for 8 days at 28 ± 2 °C (with a 12-h day length provided by fluorescent lights; 120 µ mol m^{-2} s^{-1}). At the end of the incubation, the disease symptoms of charcoal rot (black-coloured infection and microsclerotia on the root surface) in the 0–4 rating scale (0 = no visible charcoal rot symptom, while 4 = maximum disease symptoms; Nene et al. 1981) were recorded. The percentage of infected roots in *Streptomyces* inoculated treatment compared with *M. phaseolina* control was also calculated.

Greenhouse trials

The selected *Streptomyces* spp. strains were evaluated for their biocontrol potential against charcoal rot of sorghum under greenhouse conditions by toothpick method as described earlier (Thakur et al. 2007; Gopalakrishnan et al. 2019). In brief, a total of seven treatments [five test *Streptomyces* strains such as CAI-17, KAI-26, KAI-27, MMA-32 and SAI-13 inoculated (individually) + *M. phaseolina* inoculated, only *M. phaseolina* inoculated (positive control) and only water inoculated (negative control)] were evaluated with ten replications. The trial was conducted in a completely randomized design. Pot mixture (2 kg) comprising of Vertisols, sand and farmyard manure (FYM) in 3:2:1 ratio was filled in 8-inch plastic pots. Seeds (seed parent 296B; acquired from cereal pathology, ICRISAT, Patancheru) were surface sterilized and soaked in test *Streptomyces* spp. spore suspension (at 10^7 cfu ml^{-1}) or in sterilized water (for a negative control) for 1 hour. The treated seeds were sown (three pot^{-1}) immediately at 3 cm depth and thinned to one plant pot^{-1} after germination. Booster doses of test strains (5 ml $seedling^{-1}$, 10^7 cfu ml^{-1}) were applied by the soil drench method at 15, 30 and 45 days after sowing (DAS). At 10 days after 50% flowering, all the plants (except the ones in negative control) were artificially inoculated by inserting toothpick infested with the inoculum of *M. phaseolina* into the second internode of the stalk. At harvest, the disease severity was recorded by measuring the number of internodes infected and the length of infection. The trial was repeated to confirm the results.

Biochemical analysis

At harvest, the shoot samples of the five *Streptomyces* strains treated plants and both positive and negative controls were collected in liquid nitrogen and stored at − 80 °C until analyzed for antioxidant enzyme analysis. For this, the soluble proteins of the leaf samples were extracted with 0.1 M phosphate buffer, pH 7.5 containing 1% polyvinylpyrrolidone, 1 mM ETDA and 10 mM mercaptoethanol in a pre-chilled mortar and pestle. For ascorbate peroxidase activity, the extraction buffer is supplemented with 1 mM ascorbic acid. The homogenate was centrifuged at 10,000 × *g* for 10 min at 4 °C and the supernatant was stored at − 80 °C until further analysis. The antioxidant traits studied include superoxide dismutase (Martinez et al. 2001), catalase (Aebi 1984), ascorbate peroxidase (Nakano and Asada 1981), guaiacol peroxidase (Srivastava and Van Huystee 1977), glutathione reductase (Schaedle and Bassham 1977), phenylalanine ammonia-lyase (Brueske 1980), polyphenol oxidase (Gauillard et al. 1993) and total phenolic contents (Singh et al. 2013). The percent increase of the antioxidant enzymes in

Streptomyces treated plants over the positive control (*M. phaseolina* treated) were calculated.

Scanning electron microscopy (SEM)

At harvest, the shoot samples of test strains treated plants and controls were examined by SEM analysis for colonization of *Streptomyces* strains and any morphological changes occurred (Bozzola and Russel 1999; Alekhya et al. 2016). SEM analysis was done at the University of Hyderabad (UoH), Telangana, India. The morphological changes and the size of the cells were measured under SEM (Model: JOEL-JSM 5600) at the required magnifications using standard procedures at UoH Lab.

Field trials

The selected five *Streptomyces* strains were also evaluated for their biocontrol efficacy against charcoal rot of sorghum under field conditions at ICRISAT, Patancheru (17°30′ N; 78°16′ E; altitude 549 m), during 2017–18 and 2018–19 cropping seasons by toothpick inoculation method, as described in greenhouse screen. During the cropping seasons, a maximum temperature range of 28.2–37.8 °C and a minimum temperature range of 11.1–17.6 °C was recorded. Soils at the experimental site are classified as Vertisols (fine montmorillonitic isohyperthermic typic pallustert) having 52% clay, 21% silt and 26% sand, with an alkaline pH of 7.7–8.3 and an organic carbon content of 0.4–0.6%. The mineral content of the top 15 cm of rhizosphere soil includes 24.7 mg kg^{-1} soil of available nitrogen, 8.6 mg kg^{-1} soil of available phosphorous and 298 mg kg^{-1} soil of available potassium.

Sorghum seeds (variety 296B) were sown in the field at the depth of 5 cm. A total of seven treatments [five strains of *Streptomyces* spp. + *M. phaseolina* inoculated, only *M. phaseolina* inoculated (positive control) and only water inoculated (negative control)] were evaluated. For the first five treatments, the *Streptomyces* strains were subjected to seed bacterization (10^8 CFU/mL/h) before sowing and booster doses of *Streptomyces* (5 ml $seedling^{-1}$, 10^8 cfu ml^{-1}) was applied at 15, 30 and 45 days after sowing (DAS) by the soil drench method. Plants were artificially inoculated by inserting toothpick infested with *M. phaseolina* into the second internode of the stalk at 10 days after 50% flowering. The experiment had three replications in a randomized complete block design (RCBD). The plot size was three rows of 2 m long with a row spacing of 75 cm and a plant-to-plant spacing of 10 cm. After harvest, the disease severity was recorded by measuring the length of infection and the number of internodes infected.

Data analysis

The data were analyzed statistically by ANOVA (SAS 9.4 software, SAS Institute, Inc. 2018) to compare the efficiency of the selected five strains of *Streptomyces* spp. and post hoc analysis for significant differences between means was carried out using Tukey's HSD test at $P = 0.05$ (Tukey 1977). Later, the greenhouse sorghum anti-oxidant responses towards the five strains of *Streptomyces* spp. were analyzed through principal component analysis (PCA) using R-4.0.0 statistical package (R Core Team 2020).

Results

In vitro and in vivo antagonistic activity of *Streptomyces* spp.

The selected five strains of *Streptomyces* spp. inhibited *M. phaseolina* in both dual culture and metabolite production assays. All five strains recorded inhibition of > 14 mm in the dual culture assay and > 63% in the metabolite production assay, where only the organic fraction inhibited the pathogen. In the in vivo blotter paper assay, very little disease symptoms (score 1 in 0–4 scale) and lesser root infections (10–20%) were observed in *Streptomyces* spp. treated roots whereas in the *M. phaseolina* inoculated control, the highest disease severity (score 4 in 0–4 scale) and root infection (100%) were observed (Table 1; Fig. 1).

Greenhouse and field trials

Under both greenhouse and field conditions, when the five strains of *Streptomyces* spp. were evaluated for their antagonistic potential, the charcoal rot disease severity was reduced significantly when compared to the positive control (only *M. phaseolina* inoculated) (Tables 2, 3; Fig. 2). Under greenhouse conditions, the charcoal rot was observed in only 1–2.3 internodes in 2017 and 1.3–2.0 internodes in 2018 in the *Streptomyces* treated plants when compared to the positive control, where four internodes were found infected in both the years. In the field, the charcoal rot was observed in 3.2–4.3 internodes in 2017, and 2–3.1 internodes in 2018 in the plants treated with different *Streptomyces* strains when compared to the positive control, where up to 5.6 internodes were found infected (Fig. 2).

The *Streptomyces* treated plants exhibited reduced length of infection (39–81% in 2017 and 64–77% in 2018) compared to only *M. phaseolina* inoculated positive control plants in both greenhouse and field experiments. Under greenhouse conditions, the length of charcoal rot infection noted was only 2.3–7.3 cm in year 1 and 4.5–7.0 cm in year 2 in the plants treated with *Streptomyces* strains compared

Table 1 In vitro and in vivo antagonistic activity of five strains of *Streptomyces* spp. against *M. phaseolina*

Strains	Dual culture assay@	Metabolite production assay#	Blotter paper assay	
			Visual rating	Root infection (%)
S. albus CAI-17	17.0[b]	73.7[b]	1	10
S. griseus KAI-26	15.3[ab]	63.0[c]	1	20
S. albus KAI-27	14.0[a]	71.0[b]	1	10
S. griseus MMA-32	17.0[b]	71.0[b]	1	10
S. cavourensis SAI-13	15.0 [a]	77.0[a]	1	10
M. phaseolina	–	0	4	100
Control	–	0	0	0
Mean	16.0	50.8	1	23
SE ±	0.035	0.047	0	0
LSD (5%)	0.114***	0.145***	0	0
CV%	3.9	2.1	0	0

***, statistically significant at 0.001; @, zone of inhibition in mm; #, percent inhibition of organic fraction; SE, standard error; LSD, least significant differences; CV, coefficient of variation. Means having the same letter in each strains do not differ significantly using Tukey's test at $p > 0.05$

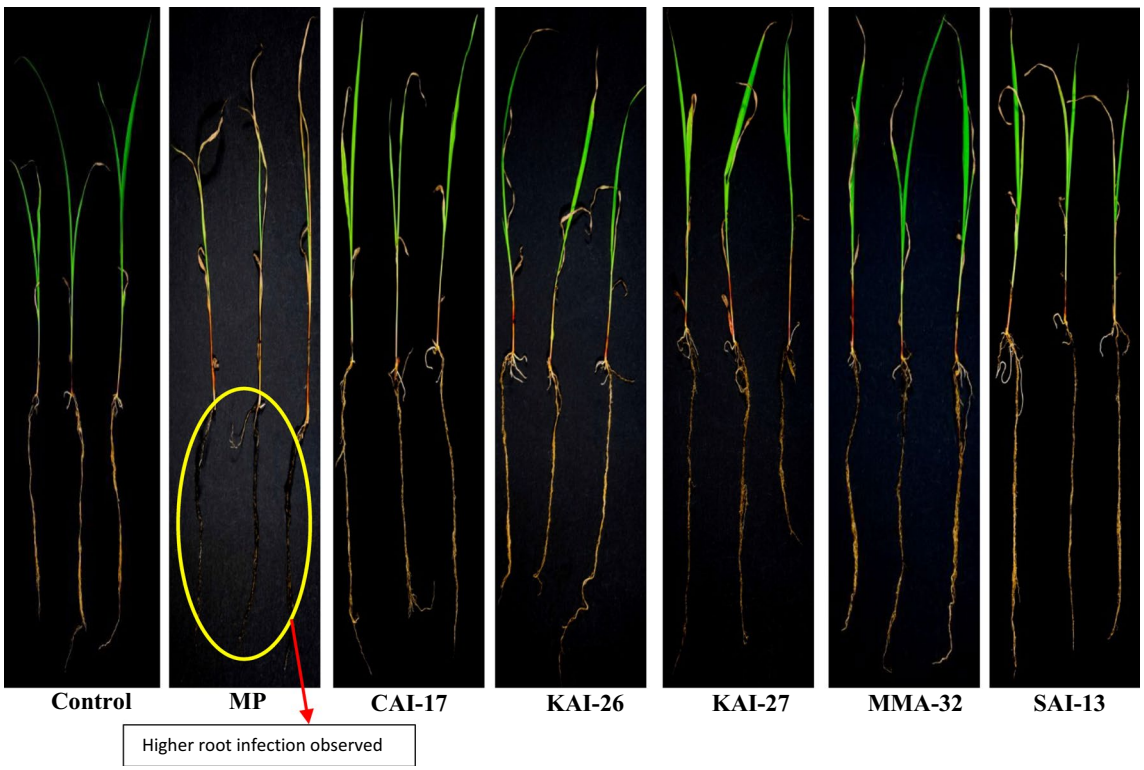

Fig. 1 In vivo antagonistic activity of five strains of *Streptomyces* spp. against *M. phaseolina* (MP) by blotter paper assay

to the positive control, where the length of infection was 12.0 cm and 19.5 cm, respectively, in year 1 and year 2. In the field experiment, the length of charcoal rot infection was 10.1–14.7 cm in year 1 and 4.1–5.7 cm in year 2 in *Streptomyces* treated plants when compared to the positive control, where the length of infection was 19.5 and 6.7 cm, respectively, in year 1 and year 2. Under greenhouse conditions, in the mean comparison of the number of internodes infected and the length of infection, all the strains were significantly better compared to the positive control (*M. phaselolina*) in both the years except MMA-32 in 2017 (Table 2). Based on the 2017 greenhouse screen, CAI-17

Table 2 Analysis of variance of the five strains of *Streptomyces* spp. for their antagonistic potential against *M. phaseolina* under greenhouse conditions

Strains	2017 (year 1)		2018 (year 2)	
	Number of internodes	Length of infection	Number of internodes	Length of infection
S. albus CAI-17	1.00^{b}	2.30^{c}	1.50^{b}	7.00^{b}
S. griseus KAI-26	1.00^{b}	3.10^{bc}	2.00^{b}	6.25^{b}
S. albus KAI-27	1.00^{b}	2.33^{c}	1.50^{b}	5.25^{b}
S. griseus MMA-32	2.33^{ab}	7.33^{ab}	1.75^{b}	5.50^{b}
S. cavourensis SAI-13	2.00^{b}	4.03^{bc}	1.25^{b}	4.50^{b}
Control (+ve)	4.00^{a}	12.00^{a}	4.00^{a}	19.50^{a}
Grand total	1.89	5.18	2.00	8.00
Tukey HSD	1.91	4.7042	1.1097	6.7934

Means having the same letter within column do not differ significantly using Tukey's test at $P=0.05$

and KAI-27 were found to be the best treatments in terms of reduction of length of infection. Under field conditions, strains CAI-17, KAI 27, and MMA-32 were found most effective in reducing length of infections in the first year. Whereas, no significant difference was observed for the internodes infected and the length of infections in year 2 compared to control (Table 3).

Biochemical analysis

Under greenhouse conditions, shoot samples treated with the five strains of *Streptomyces* spp. and *M. phaseolina* exhibited significant enhancement of antioxidants involved in the host defense response such as superoxide dismutase (up to 23%), catalase (up to 86%), ascorbate peroxidase (up to 36%), guaiacol peroxidase (up to 67%), glutathione reductase (up to 57%), phenylalanine ammonia lyase (up to 59%), polyphenol oxidase (up to 9%) and total phenolic contents (up to 28%) over shoot samples treated with only *M. phaseolina*. PCA for the sorghum shoot samples treated with the five strains of *Streptomyces* spp. and then inoculated with *M. phaseolina* exhibited a significant contribution of 76.7 and 14.6% variance for PC1 and PC2, respectively (Fig. 3). PCA analysis separated the five strains of *Streptomyces* into two groups, CAI-17 and MMA-32 in one group and the remaining strains KAI-26, KAI-27 and SAI-13 in another group. The responses of total phenolic contents and superoxide dismutase were found higher in CAI-17 and MMA-32 treated plants compared to the other strains of *Streptomyces*. This analysis confirms that among all the treatments, CAI-17 and MMA-32 significantly enhanced the tolerance against charcoal rot of sorghum.

SEM analysis

In the scanning electron microscopy, the phloem and xylem tissue morphology and size were found almost normal and intact in the *Streptomyces* spp. and *M. phaseolina* treated shoot samples when compared to only *M. phaseolina* treated positive control, where most of the tissues were found damaged (Fig. 4).

Discussion

Macrophomina phaseolina (Tassi) Goid., is a devastating fungal plant pathogen that causes charcoal rot disease in sorghum. This pathogen mainly utilizes the host-plant root system to spread its infection to the adjacent crops. Hence, it is necessary to employ rhizospheric plant growth-promoting rhizobacteria (PGPR) that are effective as anti-fungal agents. Various studies have reported the importance of PGPR especially *Streptomyces* in the suppression of fungal diseases (Bhattacharyya and Jha 2012; Anusha et al. 2019; Gopalakrishnan and Srinivas 2019). Therefore, in the present study, five strains of *Streptomyces* spp. (CAI-17,

Table 3 Analysis of variance of the five strains of *Streptomyces* spp. for their antagonistic potential against *M. phaseolina* under field conditions

Strains	2017 (year 1)		2018 (year 2)	
	Number of internodes	Length of infection	Number of internodes	Length of infection
S. albus CAI-17	3.37^{b}	11.13^{b}	2.00^{a}	4.06^{a}
S. griseus KAI-26	4.27^{ab}	14.67^{ab}	2.26^{a}	4.08^{a}
S. albus KAI-27	3.60^{ab}	10.57^{b}	2.78^{a}	5.44^{a}
S. griseus MMA-32	3.20^{b}	10.13^{b}	2.89^{a}	5.16^{a}
S. cavourensis SAI-13	3.80^{ab}	12.63a^{b}	2.67^{a}	5.72^{a}
Control (+ve)	5.57^{a}	19.50^{a}	3.11^{a}	6.72^{a}
Grand Total	3.97	13.11	2.62	5.20
Tukey HSD	2.0522	8.0697	1.3368	3.6207

Means having the same letter within column do not differ significantly using Tukey's test at $P=0.05$

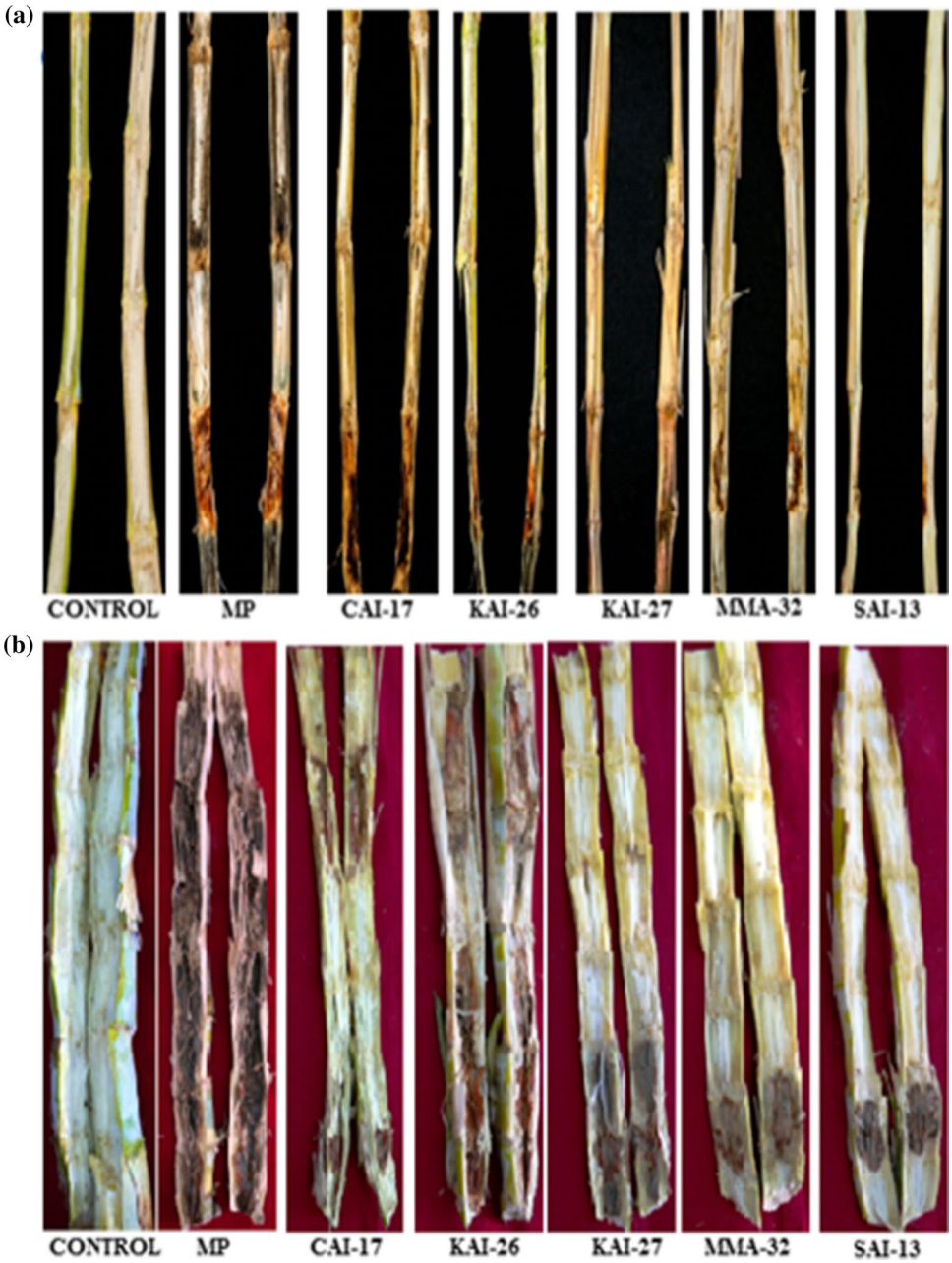

Fig. 2 Antagonistic potential of five strains of *Streptomyces* spp. against *M. phaseolina* under **a** greenhouse and **b** field conditions

KAI-26, KAI-27, MMA-32 and SAI-13) previously reported to have the capacity for plant growth-promotion in sorghum (Gopalakrishnan et al. 2011a, 2020b; Sreevidya et al. 2016) were selected to study their efficacy against charcoal rot disease.

Among the five *Streptomyces* strains studied, CAI-17 and MMA-32 consistently inhibited *M. phaseolina* followed by KAI-26, SAI-13 and KAI-27 in the dual culture, metabolite production and blotter paper assays. In the metabolite production assay, all the five strains of the *Streptomyces* exhibited a very significant antagonistic activity against the *M. phaseolina*. This could be attributed to the PGPR strains' ability to produce metabolic compounds that aid in the suppression of fungal growth (Bais et al. 2006). Often, these rhizospheric microbes individually or in consortia produce a wide variety of secondary metabolites that are known to aid in plant growth under stressful conditions (Musilova et al. 2016). Inhibition of *M. phaseolina* growth by these *Streptomyces* strains is mainly attributed to their ability to produce anti-fungal compounds such as hydrolytic enzymes and acids (Alekhya and Gopalakrishnan 2017; Vijayabharathi et al. 2018; Kim et al. 2019). These PGPR strains are mainly regarded as bio-control agents as they potentially produce chitinase, siderophore and hydrocyanic acid (HCN) which confer disease resistance in crops such as wheat (Kumar et al. 2018) and tomato (Abo-Elyousr et al.

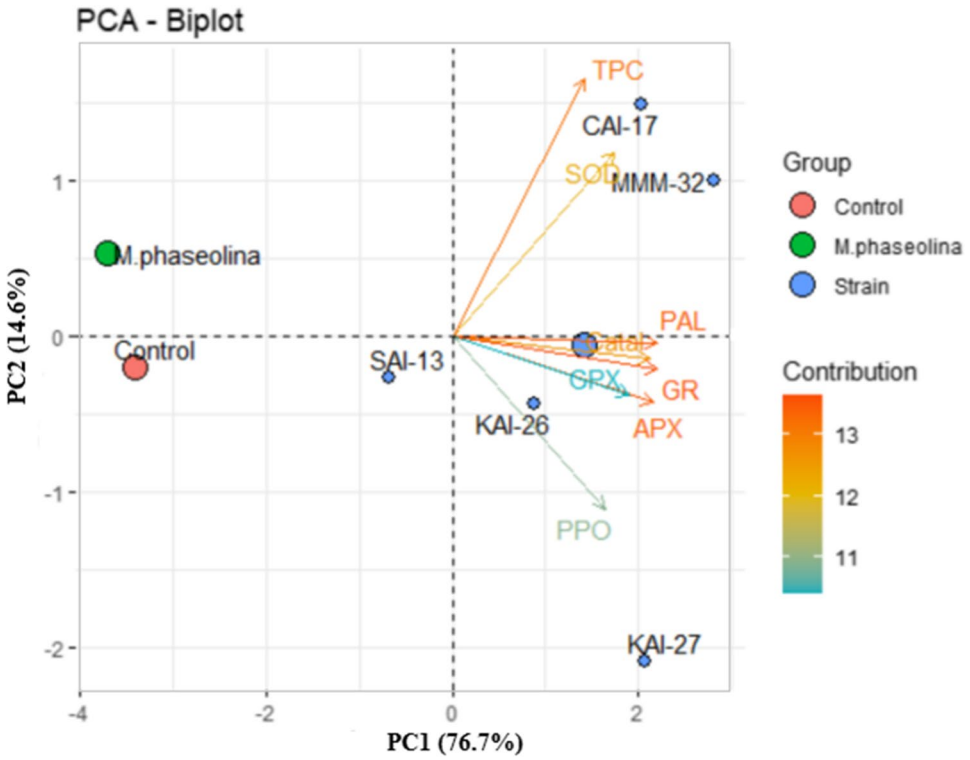

Fig. 3 Principal component analysis on sorghum antioxidant responses against *M. phaseolina* by five strains of *Streptomyces* spp. under greenhouse conditions. *SOD* superoxide dismutase, *Catal* catalase, *APX* ascorbate peroxidase, *GPX* guaiacol peroxidase, *GR* glutathione reductase, *PAL* phenylalanine ammonia-lyase, *PO* polyphenol oxidase, *TPC* total phenolic contents

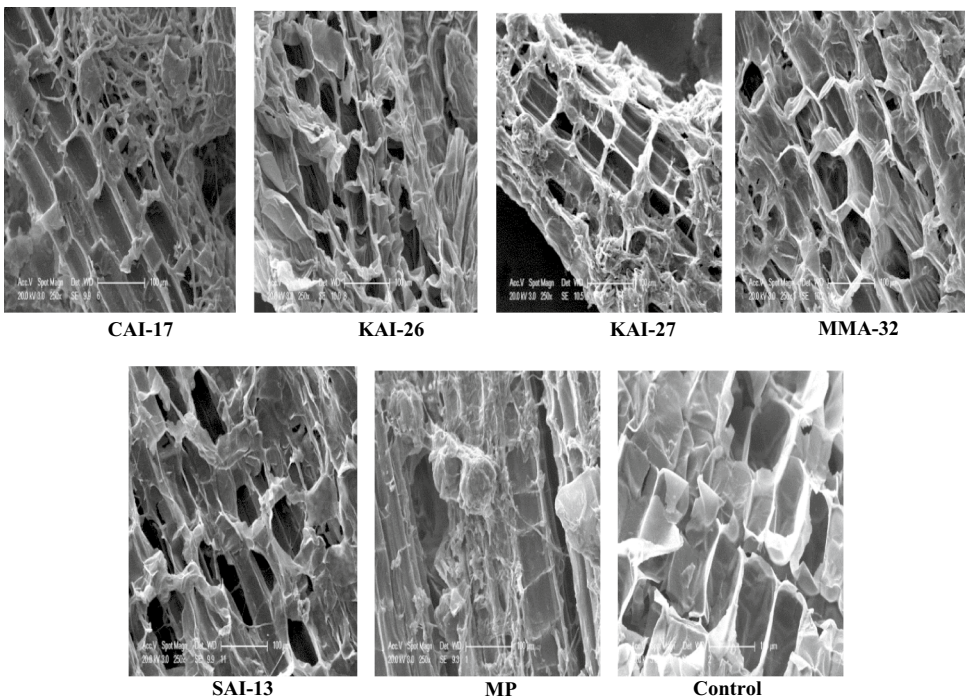

Fig. 4 SEM photographs of five strains of *Streptomyces* spp. and *M. phaselolina* (MP) showing morphological changes in the stalks of sorghum

2019). The selected *Streptomyces* strains (CAI-17, KAI-26, KAI-27, MMA-32 and SAI-13) in the present study were previously reported to produce chitinase, siderophore and HCN (Gopalakrishnan et al. 2011a; Sreevidya et al. 2016). Hydrolytic enzymes such as chitinase, siderophore and HCN were known for their ability to degrade fungal cell walls and thus help in disease suppression (Cao et al. 2005; Quecine et al. 2008). The selected *Streptomyces* strains could have inhibited the *M. phaseolina* by any one or all of these mechanisms.

In the present investigation, under both greenhouse and field conditions, the five strains of *Streptomyces* spp. significantly reduced the charcoal rot disease severity when compared to the positive (*M. phaseolina*) control. Both infected internodes (42–75 and 7–35%) and infection length (39–81 and 15–48%) were found significantly lower in greenhouse and field conditions respectively, in *Streptomyces* treated plants when compared to the positive control. However, none of strain was found significantly better compared to control in the terms of reduction in internodes infected and length of infection. High temperature and moisture stress play an important role in the development of charcoal rot disease in sorghum. In the present study, in the field trials, the charcoal rot disease severity was found more in year 1 (length of infection 10.1–14.7 cm) when compared to year 2 (length of infection 4.1–5.7 cm) in different *Streptomyces* treatments. Disease development as a whole was less in year 2 (2018) compared to year 1 (2017) as indicated by the disease severity in the only *M. phaseolina* inoculated positive control. Possible reason for this could be more rainfall and humidity and less temperature in year 2 during the disease development stage, in April. Year 1 and year 2 received an average of 3.6 and 28 mm rainfall, 56 and 70% humidity and 39 and 37 °C temperature, respectively in April. Among the five *Streptomyces* strains studied, CAI-17 and KAI-27 were found to reduce the charcoal rot disease severity the most, in both the years, than other strains in both greenhouse and field conditions. The efficacy of the selected *Streptomyces* strains was found less under field conditions when compared to the greenhouse conditions. This could be due to soil type, soil edaphic factors, environmental conditions such as temperature, humidity, and rainfall, the composition of root exudates of nearby other plant species. *Streptomyces* species generally employ an array of mechanisms such as antibiosis, site competition, HCN production, siderophore production and antifungal compounds to antagonize pathogens (Sathya et al. 2017). The five *Streptomyces* strains used in this study were earlier reported to produce siderophore, HCN and chitinase (Gopalakrishnan et al. 2011a), and hence one of these mechanisms could be the reason for their antagonistic potential against charcoal rot of sorghum. The reduction of the charcoal rot disease could also be due to enhanced biotic stress tolerance in the sorghum plants by the microbial PGPR strains, which are well known for their secondary metabolites production and root-associated hormonal signaling (Schlaeppi and Bulgarelli 2015; Egamberdieva et al. 2017). Charcoal rot of sorghum was reported to be managed by *Bacillus altitudinis*, *Brevibacterium antiquum*, *Enterobacter ludwigii*, *Acinetobacter tandoii*, *Pseudomonas plecoglossicida P. monteilii, P. chlororaphis* and *Streptomyces* spp. under greenhouse conditions (Das et al. 2018; Gopalakrishnan et al. 2011a; Alekhya et al. 2016). In the literature survey, only one study was found which reports the management of charcoal rot of sorghum under field conditions using *Amycolatopsis* sp. (Gopalakrishnan et al. 2019). However, to the best of our knowledge, there are no reports of any *Streptomyces* spp. evaluated under field conditions against charcoal rot of sorghum. Hence, based on the results of this study, it appears that the selected *Streptomyces* spp., especially strain CAI-17 and KAI-27 have the potential to manage charcoal rot in sorghum.

In the present study, under greenhouse conditions, the shoot samples treated with the five strains of *Streptomyces* spp. and *M. phaseolina* exhibited significantly enhanced antioxidant compounds including superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase, glutathione reductase, phenylalanine ammonia-lyase, polyphenol oxidase and total phenolic contents over shoot samples treated with only *M. phaseolina*. Numerous studies have reported a significant increase in the antioxidant enzymes and total phenolic contents in crops when beneficial microbes including *Streptomyces* were introduced to fight off the pathogen infection (Singh and Gaur 2016; Singh et al. 2016; Rais et al. 2017; Vijayabharathi et al. 2018; Gopalakrishnan et al. 2019). Therefore, it appears that the five strains of *Streptomyces*, CAI-17, KAI-26, KAI-27, MMA-32 and SAI-13, enhanced the expression of defence-related enzymes to combat against the *M. phaseolina* infection.

Charcoal rot, which is a serious disease in post-rainy sorghum plants is mainly characterized by the rotting of the stalks. The fungi *M. phaseolina* infects the plant tissue and discoloration of stalks is observed. In the present study, a significant reduction in the discoloration of stalks was observed in the *Streptomyces* treated sorghum stalks. Further, in the scanning electron microscopy, the phloem and xylem tissues morphology and size were found almost normal and intact in the *Streptomyces* spp. and *M. phaseolina* treated stalks. This implies a decreased colonization of *M. phaseolina* fungal bodies in the *Streptomyces* inoculated sorghum plants. This result supports the phenomenon that the PGPR which mainly enhances plant growth through root colonization, also exhibit indirect benefits of disease suppression (Vejan et al. 2016). In turn, this justifies the role of PGPR in the plant defence system under various biotic stress conditions (Hassan et al. 2019; Khan et al. 2020). This study merits a further investigation of the validation of the selected strains with diverse sorghum cultivars to understand the differential genotypic response and level of resistance induced.

The evidence obtained through this study indicates that among the five strains of *Streptomyces* spp., *S. albus* CAI-17, and *S. albus* KAI-27 exhibited significant biological control of charcoal rot caused by the *M. phaseolina* in the sorghum plants, under both greenhouse and field conditions. This strongly implies the importance of the *Streptomyces* spp. as an important tool in controlling the plant fungal pathogens even under varied environmental conditions. In

the absence of a high level of genetic resistance in high-yielding varieties, these *Streptomyces* strains could be effective in controlling charcoal rot disease and related losses in grain and stover quality of sorghum. The antifungal activity of the five *Streptomyces* strains demonstrates the multiple mechanisms of action (antibiosis, HCN, siderophore and chitinase) and hence may involve more than one antifungal metabolite. Hence, these *Streptomyces* strains are likely to be the potential candidates for the discovery of novel secondary metabolites for various biocontrol applications. Determination of the exact mechanisms of action of these *Streptomyces* strains can assist in furthering the use of eco-friendly biofungicides. Further, more evaluations of such *Streptomyces* strains must be done to assess their performance on different sorghum varieties for their capacity as growth promoters and to enhance stress tolerance.

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