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Bacteria as potential biocontrol agents for managing purple witchweed (*Striga hermonthica*) in grain sorghum

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Research Article

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Abstract

Purple witchweed [*Striga hermonthica* (Delile) Benth.], a highly destructive parasitic weed, poses a significant threat to sorghum [*Sorghum bicolor* (L.) Moench] cultivation. This hemiparasitic plant intrudes its root system into the host plant, leading to substantial yield losses, particularly in susceptible genotypes. In the pursuit of eco-friendly solutions, the biocontrol approach has gained attention as a potential management strategy for *Striga*. In this study, 13 bacterial strains belonging to the genera *Bacillus*, *Gluconobacter*, *Pseudomonas*, and *Streptomyces* were investigated in vitro for their efficiency in controlling the early-stage development of *Striga*. Among the tested strains, *Streptomyces morookaensis* NRRL B-12429 demonstrated significant inhibition of *Striga* seed germination and radicle elongation at 54.36% and 61.84%, respectively, when applied to preconditioned seeds with a synthetic germination stimulant. The effect of *S. morookaensis* on the inhibition of *Striga* seed germination was more pronounced in the presence of the host plant, sorghum, at 62.35%. However, biopriming of sorghum seeds with *S. morookaensis* did not enhance the inhibitory effects on *Striga* seed germination but resulted in a greater reduction in radicle elongation at 74.64% compared with non-primed seeds. Additionally, the biopriming with *S. morookaensis* promoted the growth of shoots and roots of germinating sorghum, regardless of the presence of *Striga* seeds. These findings highlight the potential of *S. morookaensis* strain NRRL B-12429 as a viable candidate for biocontrol agent applications in sorghum cultivation. Further exploration and investigation of its biocontrol capabilities can provide valuable insights for sustainable management practices against *Striga* infestations.

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is a C_4 grass crop with high drought, heat, and salinity tolerance. It is considered a top 10 global crop in terms of acreage and is mainly utilized for human food, animal feed, forage, and fodder. Sorghum is also an important source of fiber and feedstock for biofuel production (Gladman et al. 2022; Noort et al. 2022). Sorghum is grown on different continents, including North America, Africa, Asia, and Australia (Ostmeyer et al. 2022). Although wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) remain essential components of diets in Asia, sorghum is considered among the important staple food in Africa (FAO 2021). Millions of people in the semiarid tropic of Africa rely on sorghum as their primary source of food (Abay et al. 2022; Gwatidzo et al. 2020). However, sorghum production in sub-Saharan Africa is highly affected by purple witchweed [*Striga hermonthica* (Delile) Benth.], a root-parasitic weed (Begna 2021). *Striga hermonthica* (Striga) causes sorghum grain yield losses ranging from 20% to 80% in Africa that can reach 100% under severe infection, resulting in field abandonment, which further complicates food security (Gwatidzo et al. 2020; Yilma and Bekele 2021).

Striga hermonthica, belonging to the Orobanchaceae family, is one of the most noxious parasitic weeds within the *Striga* genus. It is an obligate hemiparasite that attacks the host plant by attaching a small sucker root system to the host plant root (Osman et al. 2023; Sibhatu 2016).

Striga hermonthica spreads rapidly due to its ability to produce 10,000 to 500,000 seeds per plant, which can survive in soils for 15 to 20 yr under optimum conditions (David et al. 2022). To germinate, the dormancy of *S. hermonthica* seeds must first be disrupted by a warm and moist stratification period (conditioning period) of around 10 d. Following the conditioning period, the seeds will germinate in response to germination stimulants present in root exudates of both host and non-host plants. The stimulants include dihydroquinones, ethylene, and sesquiterpene lactones (Mwangangi et al. 2021). Sorghum secretes strigolactone into the soil as one strategy to counter abiotic stress by promoting spore germination and hyphal branching of arbuscular mycorrhizal fungi (Kawa et al. 2021). However, strigolactone also triggers the germination of *Striga* seeds, leading to increased opportunity for *Striga* infestation in poor-fertility soil (Kawa et al. 2021; Kountche et al. 2019). The germinated *Striga* seeds will form a host–parasite attachment with the roots of their hosts, depriving the host plants of water, carbon, and essential nutrients (Stanley et al. 2021).

Several methods for controlling *Striga* have been applied and adopted, including the use of resistant or tolerant host varieties, crop rotation, application of soil enhancers, soil fumigation, hand pulling of emerged *Striga*, and using biological control agents (BCA) (Osman et al. 2023; Sibhatu 2016). Among those methods, using BCA is the most promising approach for managing *Striga* infestation because of their specific mode of action, environmental friendliness, and cost-effectiveness. In addition, the increasing awareness in weed control of targeting only unwanted species and conserving environmentally sensitive or degradation-prone areas for healthier and sustainable cropping systems supports the use of BCA to control *Striga*, particularly for resource-poor farmers (Bekele 2020; Osman et al. 2023). BCA can affect *Striga* directly by interfering with its life cycle or indirectly by affecting soil nutrient availability, changing plant physiology and root signals, or inducing resistance in the host plants against *Striga* infections (Masteling et al. 2019). Various bacterial genera have been reported to control *Striga* at varying efficiency levels, such as *Bacillus*, *Pseudomonas*, *Azospirillum*, *Burkholderia*, and *Streptomyces* (Masteling et al. 2019; Mounde et al. 2020; Oyi and Frank 2020). However, considering the huge potential of BCA and their various modes of action, studies on the bioherbicidal potential of microbes on *Striga* are still relatively limited.

This study aimed to investigate the effects of several beneficial bacteria on the early development stage of *Striga* seeds. The direct bioherbicidal effects of the selected beneficial bacteria on *Striga* seed germination and radicle elongation were screened in vitro in response to synthetic germination stimulant rac-GR24 and in planta in the presence of the host plant, sorghum. To investigate the indirect effects of the beneficial bacteria on sorghum in the early growth stage and on *Striga* seed germination, the bacterial candidate with the highest bioherbicidal activity was used to prime the sorghum seeds before germination in the presence and absence of *Striga*.

Materials and Methods

Biological Materials

Bacterial strains used in this study were *Bacillus amyloliquefaciens* NRRL B-942, *Bacillus atrophaeus* NRRL B-363, *Bacillus subtilis* NRRL B-14322, *B. subtilis* NRRL B-1471, *B. subtilis* NRRL B-59273, *Bacillus velezensis* NRRL B-1580, *B. velezensis* NRRL B-23789, *Pseudomonas fluorescens* NRRL B-2322, *P. fluorescens* NRRL B-1029, *Gluconobacter asaii* NRRL B-4241, *Gluconacetobacter diazotrophicus* PA1 5, *Gluconacetobacter xylinus* NRRL B-758, and *Streptomyces morookaensis* NRRL B-12429. All bacterial strains were selected randomly from the Agricultural Research Service Culture Collection (Northern Regional Research Laboratory [NRRL]), Peoria, IL, USA. However, they all have been reported as having beneficial effects on other plants or disease-suppression effects against various pathogens.

Striga hermonthica seeds were collected from *Striga*-infected sorghum fields at Gezira station, Sudan, in 2019. The seeds of sorghum cultivar 'Abu70' were obtained from the Arab Sudanese Seeds Company, Khartoum, Sudan, and used as a host crop in this study.

Bacterial Culture and Inoculum Preparation

Luria-Bertani agar and broth (Miller) (Nacalai Tesque, Kyoto, Japan) were used for growing *Bacillus* spp. and *Pseudomonas* spp. Mannitol agar and broth (yeast extract, 5.0 g; peptone, 3.0 g; mannitol, 25.0 g; distilled water, 1,000 ml with or without agar, 15.0 g, pH 6.5) were used to grow *Gluconacetobacter* spp. Starch casein broth and agar (soluble starch, 10.0 g; casein, 0.3 g; KNO_3, 2.0 g; NaCl, 2.0 g; K_2HPO_4, 2.0 g; $MgSO_4{\cdot}7H_2O$, 0.05 g; $CaCO_3$, 0.02 g; $FeSO_4{\cdot}7H_2O$, 0.01 g; distilled water, 1,000 ml; agar, 20.0 g agar or without agar; pH 7.0) were used to grow *Streptomyces morookaensis*. All bacterial strains were incubated at 30 C for 24 h, except *S. morookaensis,* which was incubated for 5 d. A serial 10-fold dilution was prepared from the stock culture, and a third dilution with colony-forming units per milliliter (CFU ml^{-1}) ranging from 10^5 to 10^6 was used in all the experiments.

Surface Disinfection of Plant Materials

Striga seeds were surface disinfected using ethanol and sodium hypochlorite (NaOCl) according to the method described by Daffalla et al. (2014). The seeds were soaked in 70% ethanol for 2 min and rinsed three times with sterilized double-distilled water (ddH_2O). Then the seeds were submerged in 1% NaOCl solution for 3 min with continuous stirring and thoroughly washed with ddH_2O six times. Floating seeds and debris were thrown away. The remaining seeds were placed on sterile filter paper and air-dried in a laminar flow hood without UV before being stored in sterilized vials at room temperature until used. Sorghum seeds were surface sterilized with 1% NaOCl for 6 min and thoroughly rinsed with sterile ddH_2O six times. Unless otherwise stated, all filter papers used in this study were Whatman No. 4 (Cytiva, Marlborough, MA, USA).

Conditioning of Striga Seeds

The Whatman (GF/C) glass fiber filter papers (Cytiva) were cut into 8-mm-diameter disks and placed on two layers of 90-mm filter paper in a 90-mm petri dish. For conditioning with water, the filter papers were moistened with 5 ml of sterile ddH_2O. Approximately 25 *Striga* seeds were sprinkled on each disk. Subsequently, the petri dish containing the disk was sealed with Parafilm® (Evergreen Engineering & Resources, Selangor, Malaysia), covered with a black plastic bag, and incubated at 30 C for 8 d (Gafar et al. 2015). *Striga* seeds were treated with 5 ml of the third dilution of bacterial culture instead of water for conditioning with bacterial culture and incubated as described above.

Screening of Bacterial Strains for Inhibitory Activities against Striga

Bacterial cultures were applied during conditioning as described above or as a treatment for water-conditioned *Striga* seeds. For the

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latter, the discs containing water-conditioned *Striga* seeds were dried off from excessive moisture on a filter paper and transferred to a new petri dish lined with another filter paper. Five milliliters of the third dilution of a bacterial culture were added to the petri dish and incubated for 24 h at 30 C in the dark. The next day, the bacterial-treated disks were dried of excessive moisture and transferred to another petri dish with a strip of moistened filter paper in the middle to maintain moisture. A total of 20 µl of the synthetic germination stimulant, rac-GR24 (Chiralix, Nijmegen, Netherlands) at 0.034 µM, was added to each disk. The setup was incubated at 30 C for 24 h in the dark. Afterward, the germinated seeds were observed using a trinocular stereomicroscope, and the germination data were recorded. The radicle elongation from the germinated seed was measured using S-EYE microscope camera software (Setup-1.6.0.11, Hayear Electronics, Shenzen, China). The percentage of *Striga* seed germination (GR) was calculated using Equation 1 (Kountche et al. 2019):

$$GR\% = (N_{gs}/N_{ts}) \times 100 \quad [1]$$

where N_{gs} is the number of germinated seeds per disk, and N_{ts} is the total number of seeds per disk.

The inhibition of germination percentage (IGP) was calculated according to Equation 2 (Gao et al. 2021):

$$IGP = \frac{(GPcontrol - GPtreatment)}{GPcontrol} \times 100 \quad [2]$$

where GP control is the germination percentage of *Striga* seeds treated with uninoculated culture medium as control, and GP treatment is the germination percentage of *Striga* seeds treated with bacterial culture. The same formula was used to calculate the radicle elongation in germinated seeds and the inhibition percentage of radicle root elongation by replacing the germination data with the radicle length. The experiment was performed in six replicates.

In Vitro Striga Seed Germination Assay against Selected Bacterial Strains in the Presence of Sorghum

Bacterial strains showing the highest inhibition *of Striga* seed germination and radicle elongation during the screening stage (*B. atrophaeus*, *B. velezensis* NRRL B-1580, *G. asaii*, and *S. morookaensis*) were selected for the *Striga* seed germination assay in the presence of the host plant, sorghum. The sorghum seeds were pre-germinated on two layers of moistened filter paper in petri dishes. The petri dishes were covered with a black plastic bag and incubated at 30 C for 24 h. The *Striga* seed germination assay was carried out according to the method described by Mohamed et al. (2010) with some modifications. For this germination assay, the *Striga* seeds were either preconditioned in water or conditioned in bacterial culture or only in culture medium (control). The seeds (water conditioned and unconditioned) were sprinkled separately on 0.5% semisolid water agar in petri dishes. To condition the seeds with bacterial culture or only the culture medium, the solutions were mixed with 0.001% water agar before being poured on top of the *Striga* seeds and left to solidify. The water-conditioned seeds were topped with only 0.001% water agar. After that, one germinated sorghum seed was placed at the edge of each petri dish, and the radicle was allowed to penetrate the gel. The petri dishes were then covered and incubated at 30 C for 7 d in the dark. A total of three replicates were set up for each treatment. *Striga* seed germination and radicle elongation were observed and calculated as described previously.

In Vitro Germination of Bioprimed Sorghum Seeds

Surface-disinfected sorghum seeds were directly immersed in *S. morookaensis* culture or uninoculated culture medium as a control for 1 h, then dried on sterile filter paper in a laminar-flow cabinet without UV for 3 h. Subsequently, the seeds were transferred to a petri dish lined with a moistened double layer of filter paper. Then, the petri dish was covered and placed in a dark chamber incubator at 30 C for 24 h for seed germination. The primed sorghum seeds were used in the germination assay as described above, using only water agar to cover the water-conditioned *Striga* seeds. The experiment was performed in triplicate.

Statistical Analysis

Data collected from each experiment were analyzed with the SPSS software package (v. 25.0, IBM, Armonk, NY, USA). Statistically significant differences between the mean values were evaluated by one-way ANOVA. The means were further analyzed using Tukey's test at a 95% confidence interval.

Results and Discussion

Effects of Bacterial Strains on *Striga* Seed Germination and Radicle Elongation in Response to Synthetic Germination Stimulant rac-GR24

The inhibitory effects of 13 bacterial strains on germination and radicle elongation were assessed on water-conditioned *Striga* seeds or as a treatment during the conditioning period. Regardless of the treatment conditions, all bacterial strains significantly reduced *Striga* germination. The inhibitory effects were more prominent when the bacteria were applied to water-conditioned seeds compared with direct application during the conditioning period. In *Striga*, the initial conditioning period is similar to the imbibition phase in non-parasitic plants (Yap and Tsuchiya 2023). The seed permeability and the imbibition rate are influenced by seed coat pigmentation, whereby seeds with less pigmentation have faster water uptake (Waskow et al. 2021). Bar-Nun and Mayer (2002) reported that conditioning of Egyptian broomrape (*Orobanche aegyptiaca* Pers.) seeds in water increased the permeability of the cell wall, leading to a leakage of phenolic compounds from the seeds. The seed coat of parasitic weed is usually dark and opaque and becomes permeable only when sufficient water accumulates (Daniel et al. 2013). Hence, the increased permeability of *Striga* seeds after conditioning could explain the greater inhibition of germination, as it allows more molecules to pass through compared with unconditioned seeds (Niemann 2013). In the present study, the highest percentage of germination inhibition was recorded for *S. morookaensis*, which was applied to water-conditioned seeds at 54.36%. This is followed by *B. velezensis* NRRL B-1580, *B. atrophaeus*, and *B. subtilis* NRRL B-59273 at 53.48%, 52.49%, and 50.18%, respectively (Table 1). *Streptomyces morookaensis* was among the highest inhibitors of *Striga* seed germination when applied during the conditioning period at 41.51%, with only a minor difference from *G. asaii* at 41.74%. The germination percentage for *Striga* seeds in response to synthetic germination stimulant rac-GR24 in the presence and absence of bacteria is provided in Supplementary Table S1.

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Table 1. Inhibition of *Striga* seed germination in response to rac-GR24

Bacterial strains	Inhibition of *Striga* germination[a]	
	Applied during conditioning	Applied to water-conditioned seeds
	%	
Bacillus amyloliquefaciens NRRL B-942	33.69 ± 12.3 abc	50.67 ± 4.8 a
Bacillus atrophaeus NRRL B-363	34.36 ± 5.9 abc	**52.49** ± 5.7 a
Bacillus subtilis NRRL B-14322	3.10 ± 9.5 c	39.03 ± 3.5 a
B. subtilis NRRL B-1471	22.25 ± 10.4 abc	48.93 ± 2.4 a
B. subtilis NRRL B-59273	9.39 ± 8.4 abc	**50.18** ± 9.5 a
Bacillus velezensis NRRL B-1580	38.77 ± 6.8 abc	**53.48** ± 2.5 a
B. velezensis NRRL B23789	4.55 ± 4.3 bc	45.26 ± 5.2 a
Pseudomonas fluorescens NRRL B-2322	30.04 ± 5.9 abc	34.99 ± 8.7 a
P. fluorescens NRRL B-1029	8.21 ± 11.0 abc	41.48 ± 4.5 a
Gluconobacter asaii NRRL B-4241	41.74 ± 5.0 a	48.76 ± 3.9 a
Gluconacetobacter diazotrophicus PA1 5	26.64 ± 5.0 abc	43.91 ± 8.5 a
Gluconacetobacter xylinus NRRL B-758	35.64 ± 2.9 abc	40.71 ± 7.4 a
Streptomyces morookaensis NRRL B-12429	41.51 ± 4.3 ab	**54.36** ± 1.2 a

[a]Values are the mean of six replicates; plus/minus sign (±) indicates standard error. Different letters indicate significant differences at $P \leq 0.05$, as determined by ANOVA, followed by Tukey's test. The four highest percentages of germination inhibition are in bold type.

Table 2. Inhibition of *Striga* radicle elongation in germinated seeds

Bacterial strains	Inhibition of *Striga* radicle elongation[a]	
	Applied during conditioning	Applied to water-conditioned seeds
	%	
Bacillus amyloliquefaciens NRRL B-942	35.32 ± 8.0 ab	43.48 ± 8.0 a
Bacillus atrophaeus NRRL B-363	50.03 ± 7.01 ab	**62.71** ± 3.3 a
Bacillus subtilis NRRL B -14322	31.20 ± 11.8 ab	37.17 ± 6.0 a
B. subtilis NRRL B-1471	31.51 ± 4.9 b	50.17 ± 8.0 a
B. subtilis NRRL B-59273	32.38 ± 3.9 b	44.63 ± 8.8 a
Bacillus velezensis NRRL B-1580	43.78 ± 4.7 ab	**54.92** ± 8.0 a
B. velezensis NRRL B23789	25.66 ± 5.2 b	36.83 ± 5.4 a
Pseudomonas fluorescens NRRL B-2322	29.99 ± 9.8 b	37.10 ± 10.0 a
P. fluorescens NRRL B-1029	27.45 ± 5.9 b	36.25 ± 9.1 a
Gluconobacter asaii NRRL B-4241	46.07 ± 10.8 ab	**52.60** ± 18.8 a
Gluconacetobacter diazotrophicus PA1 5	22.50 ± 6.1 b	38.84 ± 13.6 a
Gluconacetobacter xylinus NRRL B-758	40.98 ± 12.3 ab	30.59 ± 22.0 a
Streptomyces morookaensis NRRL B-12429	61.47 ± 10.9 a	**61.84** ± 14.4 a

[a]Values are the mean of six replicates; plus/minus sign (±) indicates standard error. Different letters indicate significant differences at $P \leq 0.05$, as determined by ANOVA, followed by Tukey's test. The four highest percentages of radicle elongation inhibition are in bold type.

Similar inhibitory effects were observed for *Striga*'s radicle elongation, whereby the effects of the bacterial treatment were stronger when applied to water-conditioned *Striga* seeds. The highest percentage of radicle elongation inhibition was recorded for *B. atrophaeus* at 62.71% when applied to water-conditioned seeds, followed by *S. morookaensis* at 61.84%, *B. velezensis* NRRL B-1580 at 54.92%, and *G. asaii* at 52.60% (Table 2). The *Striga* radicle elongation in response to synthetic germination stimulant rac-GR24 in the presence and absence of bacteria is provided in Supplementary Table S2. An earlier study by Neondo et al. (2017) showed that bacterial isolates belonging to *Bacillus*, *Streptomyces*, and *Rhizobium* genera caused *Striga* seed decay by producing enzymes such as xylanases and pectinase that act directly on seeds by decomposing organic matter. *Bacillus atrophaeus* is one of the *Bacillus* species known to cause decay in *Striga* seeds through the action of such compounds (Bekele 2020). Mounde et al. (2020) reported that the *Bacillus* stains in the study produce indole acetic acid (IAA), which was responsible for the inhibition of *Striga* seed germination. The inhibition mechanism of IAA could possibly be mediated through enhancing the Abscisic acid-biosynthesis pathway leading to induction of dormancy and inhibition of germination (Shuai et al. 2017).

On the other hand, the genomic analysis of *B. velezensis* NRRL B-1580 showed that it contains a specific cluster of genes related to the biosynthesis of various secondary metabolites, such as surfactin, bacillibactin, and polyketides (Rabbee et al. 2023; Zhao et al. 2017). He et al. (2022) were the first to report on the bioherbicidal potential of *B. velezensis* NRRL B-1580 through the inhibition of *O. aegyptiaca* seed germination. Although the mechanism is still unclear, the effects of the inhibition were attributed to diketo-piperazine–type metabolites from *B. velezensis* NRRL B-1580. It is tempting to speculate that the observed inhibitory effects from *B. velezensis* NRRL B-1580 in the current study could also be related to the same type of metabolites. *Gluconobacter asaii* is an acetic acid bacterium that produces acetic acid during sugar fermentation. Previously, Gafar et al. (2018) reported a significant inhibitory effect of acetic acid on *Striga* seed germination. Acetic acid is a contact herbicide and recommended as an environmentally friendly control method for weeds (Duke et al. 2022; Owen 2002; Webber et al. 2018). Another possible explanation for the inhibitory effect of *G. asaii* is via IAA, as *Gluconoacetobacter* spp. are known to produce a high level of IAA (AbdelRazek and Yaseen 2020).

Streptomyces is a major group of soil microbes with potent bioherbicidal activity. Bilanaphos and glufosinate ammonium are

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Table 3. Inhibition of *Striga* seed germination in the presence of sorghum

Bacterial strains	Inhibition of *Striga* germination[a]	
	Applied during conditioning	Applied to water-conditioned seeds
	%	
Bacillus atrophaeus NRRL B-363	36.02 ± 12.1 a	46.76 ± 13.5 a
Bacillus velezensis NRRL B-1580	29.86 ± 24.2 a	45.91 ± 1.8 a
Gluconobacter asaii NRRL B-4241	30.05 ± 10.72 a	59.02 ± 2.4 a
Streptomyces morookaensis NRRL B-12429	60.68 ± 7.41 a	62.35 ± 4.7 a

[a]Values are the mean of three replicates ± standard error. Same letters indicate nonsignificant differences at $P \leq 0.05$, as determined by ANOVA, followed by Tukey's test.

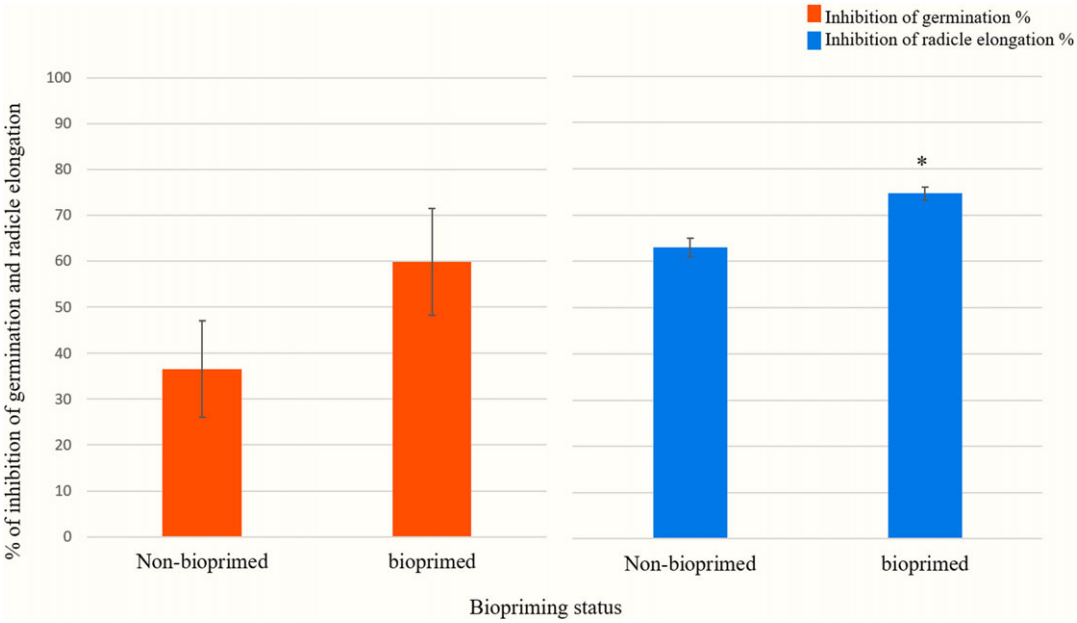

Figure 1. Biopriming of sorghum with *Streptomyces morookaensis* induced higher inhibition percentage of *Striga* seed germination and radicle elongation. Error bars indicate standard error of the mean calculated from three replicates. Asterisk indicates a significant difference at $P \leq 0.05$, as determined by ANOVA.

commercially available bioherbicides formulated using metabolites from *Streptomyces* (Bo et al. 2019). In addition, some antibiotics from *Streptomyces* have been developed as bioherbicides, including blasticidin, nigericin, hydantocidin, geldanamycin, and nojirimycin (Harada et al. 2017; Li et al. 2013; Nakajima et al. 1991; Won et al. 2016). The *Streptomyces* strain producing nojirimycin has the ability to suppress seed germination and radicle lengthening in parasitic weeds by inhibiting β-glycosidase, an important enzyme in the early stage of parasitic weed germination (Harada et al. 2017; Wakabayashi 2015). In another study, Chen et al. (2016) revealed that the culture filtrate of *Streptomyces enissocaesilis* significantly reduced the germination rate of a parasitic weed, sunflower broomrape (*Orobanche cumana* Wallr.)

In Vitro Effects of Bacterial Strains on *Striga* Seed Germination in the Presence of Sorghum

Based on screening results with rac-GR24, four bacterial strains with the highest inhibition of germination and radicle elongation percentage in water-conditioned *Striga* seeds were selected and used in the *Striga* germination assay in response to a natural germination stimulant from the host plant, sorghum. Treatment of water-conditioned seeds with bacterial culture also resulted in a higher reduction of *Striga* seed germination in comparison to the treatment applied during conditioning. *Streptomyces morookaensis* showed the highest germination inhibition percentage in both treatments (60.68% and 62.35%) (Table 3). Unfortunately, the inhibition data show no statistically significant differences between treatments. The germination percentage for *Striga* seeds in response to natural germination stimulant from the host plant sorghum in the presence and absence of bacteria is provided in Supplementary Table S3.

The widely studied *S. morookaensis* strain Sm4-1986 is reported to produce IAA and siderophores (Zhu et al. 2021). In the study, siderophores were associated with the antagonistic activity against the banana (*Musa acuminata* Colla) pathogen *Fusarium oxysporum* f. sp. *cubense*. Strikingly, the iron-binding potential from siderophores has also been manipulated to produce a broad-spectrum herbicide (Witschel 2009). The same strain also produced bioactive compounds such as harziandione, streptimidone derivative, phenole, and xerucitrinin (Wu et al. 2022). Interestingly, diterpene harziandione is also produced by *Trichoderma harzianum*, which has been shown to significantly inhibit *S. hermonthica* germination and haustorium initiation (Azarig et al. 2020). *Streptomyces morookaensis* is also known to produce antibiotic blastocidin (Nishimura et al. 1995), which is highly selective for dicots (Kao-Kniffin et al. 2013).

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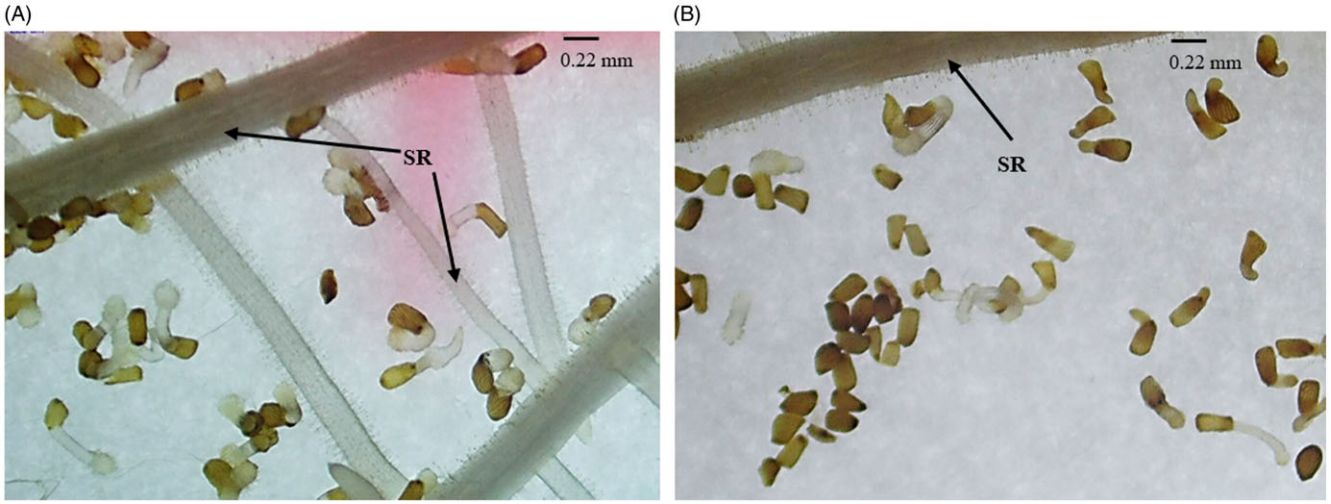

Figure 2. Elongation of *Striga* radicle in the presence of sorghum. (A) Non-bioprimed and (B) bioprimed sorghum. SR, sorghum root.

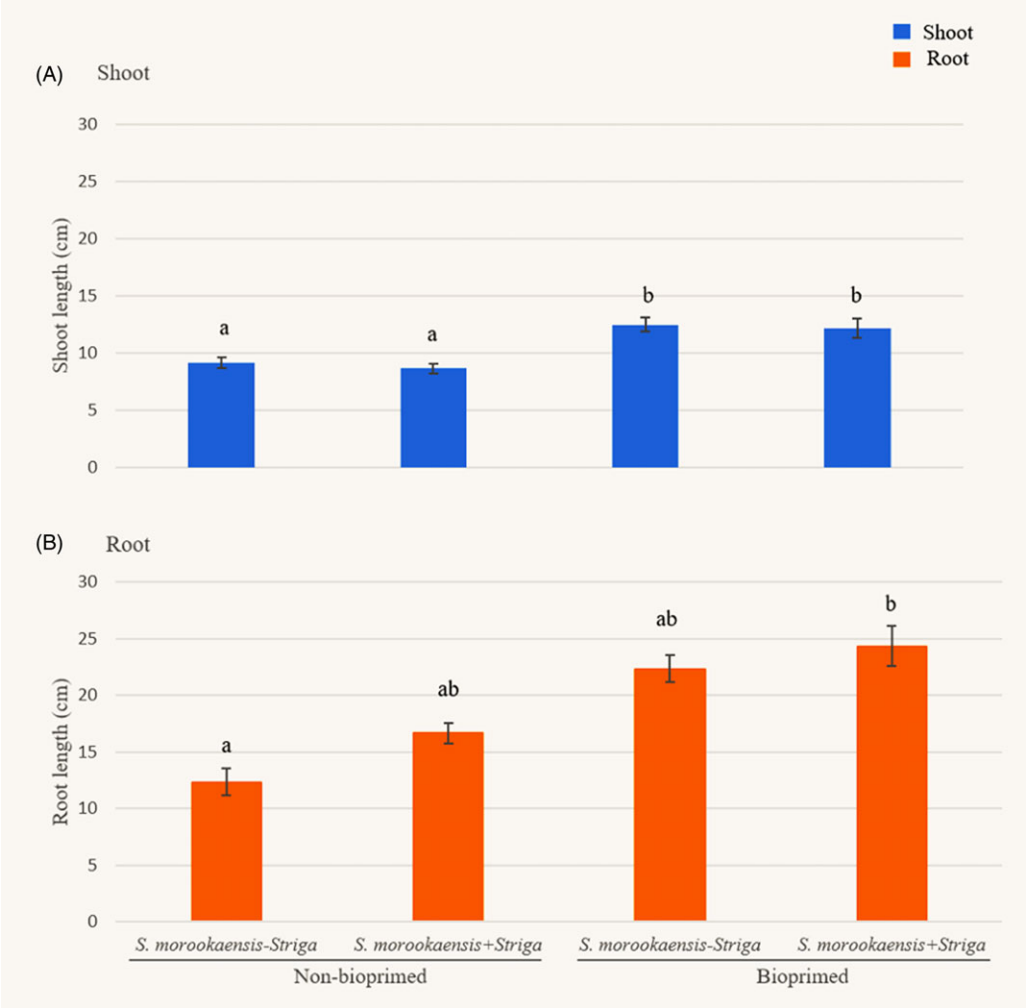

Figure 3. Sorghum biopriming with *Streptomyces morookaenis* promotes shoot and root growth. Striga represents *S. hermonthica*. Error bars indicate standard error of the mean calculated from three replicates. Different letters indicate significant differences at $P \leq 0.05$, as determined by ANOVA, followed by Tukey's test.

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In Vitro Effects of Sorghum Biopriming with Streptomyces morookaensis on Striga Seed Germination and Radicle Elongation

Sorghum seeds were bioprimed with *S. morookaensis* to investigate the potential of colonized sorghum seeds to inhibit *Striga* seed germination and radicle elongation. Figures 1 and 2 show that biopriming with *S. morookaensis* imposed a stronger inhibitory effect than when applied to *Striga* seeds directly in water agar. The inhibition of *Striga* seed germination was recorded at 59.83% with bioprimed sorghum compared with only 36.56% with the non-bioprimed sorghum. Meanwhile, the effects of biopriming on *Striga* radicle elongation differ significantly by more than 10% in the presence of bioprimed sorghum. The inhibition action may occur indirectly via reprogramming of seed germination signaling pathways, leading to reduced germination or radicle malformation.

In Vitro Effects of Biopriming with Streptomyces morookaensis on Sorghum Shoot and Root Lengths

To determine whether the colonization of sorghum seed with *S. morookaensis* was able to promote sorghum growth, the shoot and root lengths of the germinated sorghum seedlings were measured in the presence or absence of *Striga* (Figure 3). The results show that biopriming with *S. morookaensis* increased the length of shoot and root of the germinated sorghum seedlings in either condition. Interestingly, the presence of *Striga* further increased sorghum root length regardless of the biopriming treatment. Biopriming of seeds before germination allows the beneficial bacteria to enter or adhere to the seeds from the beginning. It is considered an attractive, cost-effective approach to improve seed germination rates under hostile environmental conditions and to activate plant defense mechanisms at early stages of plant development (Lastochkina et al. 2020; Mahmood et al. 2016). Furthermore, biopriming of seeds with bacteria is proven to promote crop productivity and growth (Chakraborti et al. 2022; Sharifi et al. 2011) and increases plant resilience under adverse conditions (Fiodor et al. 2023). The ability of *Streptomyces* to secrete phytohormones that stimulate plant growth has been shown for *Streptomyces* sp. CLV45 and *Streptomyces alfalfae* 11F that produce IAA and/or siderophores (Pang et al. 2022).

In summary, this study has confirmed the potential of several beneficial bacterial strains to suppress *Striga* infestation by affecting germination and radicle elongation, especially in conditioned seeds. Among the tested strains, *S. morookaenis* showed the highest reduction in *Striga* seed germination and radicle elongation regardless of seed conditioning status. The inhibitory effects were higher when the sorghum seeds were bioprimed with *S. morookaenis* before germination in the presence of *Striga*. Biopriming of sorghum seeds also promoted its growth. These findings provide significant insights into *Striga* management. In line with the goal of sustainability in weed management, the future of *Striga* management strategies is expected to rely heavily on biomolecules or microbes that can inhibit germination or suppress the growth of the parasitic plant. However, it is essential to examine the compatibility of the candidate bacteria with the host, consider a suitable inoculum medium, consistency of their inhibitory effects, and the maintenance of their activities in infested soil by conducting field experiments under different environmental conditions to ensure optimum suppression effects.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/wsc.2024.42

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Competing interests. The authors declare no competing interests.

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