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ORIGINAL ARTICLE

Selection of Soil Borne Bacteria for Suppression of *Striga Hermonthica* (Del.) Benth.

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Mohammed Mahgoub Hassan, Migdam El Sheik Abdel gani and Abdel Gabar El Tayeb Babiker: Selection of Soil Borne Bacteria for Suppression of *Striga Hermonthica* (Del.) Benth: *Adv. in Nat. Appl. Sci., 3(1): 27-34, 2009*

ABSTRACT

Witchweeds (*Striga* spp.) are important root parasites of many cereal and legume crops in savanna and Sahelian regions of Africa. Rhizobacteria strains can affect the interaction between *Striga* and cereals. The combination between bacterial strains negatively impacted on *Striga* seed germination and haustorium initiation. Combinations of bacterial strains were often more suppressive to haustorium initiation than individual isolates and strains. A combination of the bacterial strains *Azospirillum brasilense* and *Pseudomonas putida; A. brasilense* and *Azomonas spp; Azotobacter vienlandi* and *Bradyrhizobium japonicum; A. brasilense* and *Azomonas spp.* inhibited germination by 18 to 34% in comparison to the corresponding control. Individual bacteria and strains viz *P. putida, B. japonicum* and *Azotobacter* spp. inhibited germination by 2 to 10%. Some bacterial isolates and strains reduced haustorial initiation, irrespective of the haustorial inducing factors. Others were specific showing stimulant dependent inhibitory effects. Isolates G8, G37, M2 and the strains *P. putida*, *Bacillus* spp., *Azotobacter* sp. and the combination of *Bradyrhizobium* and *Azotobacter* inhibited haustorial initiation by 12 - 50% in response to DMBQ, while the isolate M20, G8C and the strains *Klebsiella* spp., *Azotobacter spp*., *Bacillus* spp.(B1) and *P. putida* inhibited haustorium initiation by 12 - 36% in response to root exudates. The bacterial isolate G11a enhanced haustorium initiation by over 30, irrespective of the haustorial initiation factor.

Key word: *Striga hermonthica*, biocontrol, bacteria, suppression, stimulation

Introduction

Sorghum (*Sorghum bicolor*) is the most important food crop in savanna areas of the West and Central Africa region, where grain yield averaged 0.71 t in 1999 (FAO, 2001). *Striga* species present major constraints for cereals and legumes production in tropical and sub-tropical Africa and the Indian subcontinent (Rao and Musselman, 1987). *Striga* life cycle is strongly cued to that of its host and to the environment featured by wet and dry seasons (Ejeta *et al*., 1993; Babiker, 2007). A freshly harvested seed does not germinate and requires an after-ripening period which extends from few weeks to several months (Joel, 1995). To germinate an after-ripened seed needs pre-treatment in a warm mist environment (conditioning) for several days and a subsequent exposure to a germination stimulant (Joel, 1995). Exposure of the seeds to germination stimulants during the conditioning period to germination stimulants delays conditioning and reduces the sensitivity of the seeds to su[[bsequent treatments with the stimulants.

Recently it was discovered that the germination strategy in *Striga* is based on ethylene biosynthesis and action (Logan and Stewart, 1991). Conditioning removes a restriction on the ethylene biosynthetic pathway in the seeds (Babiker *et al*., 2000). Germination stimulants, natural or synthetic, induce ethylene biosynthesis in *Striga* seeds (Logan and Stewart, 1991). Exposure of seeds to germination stimulants during conditioning tends

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to decrease eventual germination of the parasite (Vallance 1951; Hsiao *et al*., 1981). Logan and Stewart (1991; 1995), working with *S. hermonthica* and using sorghum root exudates, thidiazuron and GR24, proposed that stimulants induce ACC synthase, trigger ethylene biosynthesis and that the ethylene produced induces germination. Babiker *et al*., (1993; 1994), working with *S. asiatica*, proposed that germination stimulants, in addition to induction ACC synthase, increase the capacity of the seeds to convert ACC to ethylene. Subsequent to germination, which occurs in close proximity of the host roots, *Striga* germlings, in response to a second chemical signal from the host roots, produce haustoria. The haustorium, a physiological bridge between the host and the parasite, represents the switch from the vegetative to the parasitic mode of life. The haustorium attaches, penetrates and establishes connection with the host xylem. Following attachment the *Striga* seedlings remain subterranean for six to eight weeks prior to emergence (Parker and Riches, 1993).

Biological control, in broad sense, is the use of living natural enemies to control noxious pests (Evan, 1974). Microorganisms are increasingly being considered as control agents for *Striga* (Babalola, 2002; Babalola *et al*., 2003). Among the micro-organisms colonizing the root surface are bacteria of the genus *Pseudomonas* (Babalola, 2002). Work on bacteria as *Striga* suppressants was limited despite the recognized potential of such an approach and the anticipated ease of application in comparison to other biological agents (Berner *et al*., 1995; Parker and Riches, 1993). (Berner *et al*., 1999) studied the efficacy of *Pseudomonas syringae* pv. *glycinea* strains in stimulating germination of several *Striga* spp. He reported that the bacterium strain were consistently better stimulators of seeds germination than exogenously applied ethylene gas or root pieces of cowpeas. Our basic idea stems from the fact that soil microorganisms including bacteria produce a variety of phytohormones. Of these phytohormones auxins, Cytokinins and ethylene are of special relevance to *Striga* early development stages. The objective of the present study was set to identify soil borne bacteria capable of suppressing, triggering suicidal germination and/or perturbing early developmental stages in *S. hermonthica*.

Materials and methods

Striga hermonthica seeds were collected from parasitic plants growing under sorghum fields at the Gezira Research Station Farm of the Agricultural Research Corporation in Wad Medani. Seeds were surface disinfected as described by (Hassan *et al*., 2008). The seeds were stored in sterile glass vials and kept at room temperature until used.

Chemicals

Striga germination stimulant GR24 was kindly provided by Professor B. Zwanenberg, the University of Nimijhen, the Netherlands. 1-aminocyclopropane-1-carboxylic acid (ACC) was obtained from Sigma Ltd. 2, 6-dimethoxybenzoquinone (DMBQ) was kindly provided by Dr. Sugimoto, Y. from Kobe University, Japan.

Seeds of sorghum cv. Tabat (*Striga* susceptible) were surface disinfected and sowing in pot as described by Hassan *et al*., 2008. The root macerate, filtered through Whatman No. 1 filter paper, was kept in a fridge at 5 ^{0}C for not more than 2 - days. The filtrate was diluted 3- times with distilled water prior to use.

Collection and isolation of soil borne bacteria

Two hundred and two of bacterial isolates were isolated from sorghum rhizosphere soils collected from four locations namely Shambat, Gadaref, Abuharaz and Wad Medani in Sudan. In addition eight bacteria strains *Azotobacter vienlandi and Bacillus spp*. were obtained from the Faculty of Agriculture, University of Khartoum, while *Pseudomonas putida*, *Azomonas* spp., *Bradyrhizobium japonicum*, *Azospirillum brasilense, A. amazonas* and *Klebsiella planticola* were obtained from the Environment and Natural Resources Research Institute (ENRRI), the National Centre for Research, Khartoum.

Striga seeds were conditioned as described by Babiker *et al*., (1993). Briefly, the sterilized discs placed in 9 cm petri dishes lined with glass fiber filter papers (GF/C), were moistened with 5 ml distilled water, or diluted nutrient broth medium inoculated or not inoculated with the respective bacteria. The standard broth medium was inhibitory to *Striga* germination. A dilution of 10^{-8} was found to secure both adequate bacterial growth and *Striga* germination and was used in all laboratory experiments.

Effect of bacteria on GR24 -induced germination of S. hermonthica seeds

A total of 211 isolates, strains and combination of bacteria were screened in the laboratory for their ability to inhibit GR24 - induced germination of *S. hermonthica* seeds. The screening was done in two stages: a preliminary stage comprising all isolates and strains and a confirmatory stage comprising 21 selected strains, isolates and combinations. The preliminary experiment was conducted in three batches. Each batch was screened, under the same conditions on a separate day. Each experimental run included two controls comprising

S. hermonthica seeds conditioned either in sterile distilled water or diluted nutrient broth medium (conditioning medium). In both the preliminary and confirmatory screenings, *Striga* seeds were treated with aliquots (20µl) of GR24 at 0, o.o34, o.34 and 3.4 µM, reincubated and examined for germination 24 h later.

Effects of bacteria on ACC -induced germination of S. hermonthica seeds

Twenty two bacterial strains, isolates and combinations were screened for their ability to inhibit ACC-induced germination of *S. hermonthica* seeds. The conditioned were treated with aliquots (20µl) of ACC at 2.5, 5, 7.5 and 10 µM or distilled water. The seeds were reincubated and examined for germination.

Effects of bacteria on haustorial initiation in S. hermonthica

Seventeen bacterial strains and isolates (single or in combinations) were screened for their effects on haustrorium initiation. Surface sterilized *Striga* seeds, placed on 8 mm glass fibre discs conditioned in presence and absence of bacterial isolates and/or strains as described above, were dapped on filter papers (Whatman No. 1) and transferred to sterile Petri dishes. The discs containing *Striga* seeds were treated, each, with 20 µl GR24 solution (o.34 µM) to induce germination. The Petri dishes were sealed with parafilm and placed in black ploythene bags, then incubated in the dark at 30 ºC for 48h. The discs containing the germinated seeds (*Striga* germlings) dapped on a filter paper, were placed, and inverted top-down on similar discs without *Striga* seeds. The Pairs of discs were treated either with 40 µl solution of 2, 6-dimethoxybenzoquinone (DMBQ) (10 µM) or 40 µl of sorghum root macerate. *Striga* germlings resulting from seeds conditioned in nutrient broth medium or in distilled water similarly treated with DMBQ or sorghum root macerate were included as controls for comparison.

In all experiments, treatments were arranged in a randomized complete design with 4-5 replicates. Data on percentage germination and haustorial initiation were calculated for each disc, transformed to arcsine (Gomez and Gomez, 1984) and subjected to analysis of variance (ANOVA). Means were comparing with the least significance difference (LSD) at 5% level. The data were back transformed and tabulated.

Results and Discussion

Effects of bacteria on GR24 -induced germination of S. hermonthica seeds

Germination percentage of *Striga* seeds varied among germination stimulants and experimental run. Results showed that GR24 applied to seed conditioned in water induced germination by 42 - 46% (Table 1). Seed conditioned in the nutrient broth were less responsive to the stimulant, albeit not significantly. All bacterial isolates and strains were suppressive to *Striga* germination. In among all bacterial strains, B1 (*Bacillus spp.)* was the most inhibitory. *Bacillus spp*. (B2), D8, M7, M20, G37 and D50 were less suppressive. *Bacillus spp.* (B3) was the least effective at GR24 concentrations of 3.4 µM and 0.34 µM. However, at the lowest concentration of the stimulant (o.o34 µM) the isolate effectively curtailed germination as only 23 % of the parasite seeds germinated (Table 1).

Table 1: Effects of bacteria on *S. hermonthica* seeds germination

	Conditioning medium		Germination (%) Bacteria								
GR24(µM)	A[1]	B[2]	D8	M7	M20	G37	D50	B1(*Bacillus* spp.)	B2(*Bacillus* spp.)	B3(*Bacillus* spp.)	mean
3.4	(51.41)46	(48.76)44	(37.45)37	(35.92)37	(28.47)32	(32.15)34	(32.479)34	(27.15)31	(33.16)35	(40.39)39	(36.73)37
0.34	(50.28)45	(47.24)43	(37.99)38	(34.61)36	(31.24)34	(32.99)35	(36.91)37	(20.01)27	(26.29)31	(39.72)39	(35.73)37
0.034	(45.00)42	(42.14)40	(31.63)34	(30.72)34	(27.00)31	(25.14)30	(30.41)33	(13.72)22	(20.06)27	(14.92)23	(28.07)32
mean	(48.90)a44	(46.05)43	(35.69)37	(33.75)35	(28.91)33	(30.09)33	(33.27)35	(20.29)27	(26.50)31	(31.68)34	
LSD for interaction		(±6.54)									
LSD for bacteria		(±3.77)									
LSD for concentration		(±2.06)									

( ) indicates arcsine transformed data.
[1]Aqueous
[2] Nutrient broth

GR24 applied to seeds conditioned in water induced high germination (52 – 60%). The nutrient broth reduced the response of seeds to GR24 significantly at the two highest, but not at the lowest concentration in comparison to the aqueous control (Table 2). The effects of bacteria on *Striga* seeds germination varied from non- significant to highly significant when compared with the nutrient broth. G14 and *B. japonicum* were the only isolates which caused significant reductions in germination. Isolate G8 displayed inconsistent performance. It displayed high inhibition at the highest concentration of GR24, but effected non- significant inhibition at the two lower concentrations of the stimulant.

GR24 applied to seeds conditioned in water induced 40 - 48 % germination (Table 3). Seeds conditioned in nutrient broth and similarly treated with GR24 exhibited comparable germination. All bacterial combinations were suppressive to *Striga* germination. The combination between *Azomonas spp.* and *A. brasilense* was the most inhibitory and reduced germination in response to GR24 to between 32 and 33% (Table 3).

Table 2: Effects of bacteria on *S. hermonthica* seeds germination

			Germination (%)						
	Conditioning medium		Bacteria						
GR24(µM)	A[1]	B[2]	G14	*B. japonicum*	*klebsiella*	*Azotobacter*	G8	G7	mean
3.4	(73.96)59	(62.23)52	(57.44)49	(58.18)50	(57.71)49	(60.58)51	(45.21)42	(61.97)52	(59.66)51
0.34	(74.66)60	(63.56)53	(52.67)46	(52.20)46	(67.99)55	(61.77)52	(60.16)51	(60.16)51	(61.64)52
0.034	(61.70)52	(60.43)51	(48.01)44	(51.06)46	(63.80)53	(54.71)48	(58.99)50	(60.25)51	(57.37)49
mean	(70.11)57	(62.07)52	(52.71)47	(53.81)47	(63.17)53	(59.02)50	(54.78)48	(60.79)51	
LSD for interaction		(±6.23)							
LSD for bacteria		(±3.59)							
LSD for concentration		(±2.20)							

( ) indicates arcsine transformed data.
[1]Aqueous
[2]Nutrient broth

Table 3: Effects of bacteria on S. hermonthica seeds germination

			Germination (%)							
	Conditioning medium		Bacteria							
GR24 (µM)	A[1]	B[2]	Azomonas+ A brasilense	Azomonas+ B. japonicum	P. putida+ Azomonas	Klebsiella+ A.brasilene	A. brasilense+B. japonicum	P. putida+B. japonicum	P. putida+ klebsiella	Mean
3.4	(55.61)48	(50.97)46	(29.72)33	(38.60)38	(34.13)36	(36.79)37	(43.91)41	(35.82)37	(45.48)42	(41.22)40
0.34	(52.21)46	(50.50)46	(28.23)32	(44.13)42	(39.08)39	(31.40)34	(36.16)37	(39.30)39	(41.24)40	(40.25)39
0.034	(42.37)40	(42.25)40	(27.70)32	(34.02)36	(36.20)37	(30.36)33	(35.13)36	(35.65)37	(41.29)40	(36.11)37
mean	(50.06)50	(47.91)44	(28.55)32	(38.92)39	(36.47)37	(32.85)35	(38.40)38	(36.92)37	(42.67)41	
LSD for interaction			(±6.88)							
LSD for bacteria			(±3.97)							
LSD for concentration			(±2.29)							

( ) indicates arcsine transformed data.
[1]Aqueous
[2] Nutrient broth

Effects of bacteria on ACC -induced germination of S. hermonthica seeds

ACC at 10, 7.5, 5 and 2.5 µM applied to seed conditioned in water induced high germination 53, 48, 48 and 44 %, respectively (Table 4). Seeds conditioned in the nutrient broth displayed reduced germination (44 - 48 %). The depressive effects of the medium decreased with increasing ACC concentrations. All bacterial isolates and strains significantly reduced germination, in response to the highest ACC concentration (10 µM). However, at the lower concentrations of ACC variable and inconsistent responses were achieved. In among all bacterial isolates M7, D8, D46 and D50 were the least inhibitory to germination. Isolates M20, G37 and *Bacillus* spp. (B2) strain effected moderate inhibition, while *Bacillus* spp. B1 and B3 showed the highest inhibitory effects. Seeds conditioned in the presence of bacterial isolate B1 (*Bacillus* spp.) and treated with ACC at 10, 7.5, 5 and 2.5 µM displayed 46, 36, 40 and 33% germination, respectively. The corresponding germination figures for B3 (*Bacillus* spp.) were 45, 39, 34 and 39% (Table 4).

In the second batch, ACC applied to seeds conditioned in water induced 49 - 58% germination (Table 5). Seeds conditioned in the nutrient broth displayed reduced germination (46 - 56 %). The bacterial strains *B. japonicum*, *P. putida*, *A. amazonas* and *Azotobacter* showed germination comparable to the nutrient broth. Strain *A. brasilense* and its combination with *P. putida* suppressed germination significantly. The combination of the strains was more suppressive than each alone.

ACC at 2.5 - 10 µM applied to seeds conditioned in water induced 37- 47 % germination (Table 6). Seeds conditioned in the nutrient broth displayed lower germination (36 - 42%). In among the seven bacterial combinations screened, the combinations between *P. putida* and *B. japonicum*; *Azotobacter* and *B. japonicum*

and *Azomonas* and *A brasilense* were the most inhibitory. The highest inhibitory effect was achieved by the combination *P. putida* plus *B. japonicum*. The combinations *A. brasilense* plus *Klebsiella* and *Azomonas* plus *B. japonicum* were inhibitory at the lowest ACC concentration (2.5µM).

Table 4: Effects of bacteria on S. hermonthica seeds germination in response to ACC

			Germination (%)									
	Conditioning medium		Bacteria									
Treatment ACC µM	A[1]	B[2]	M7	D8	D46	D50	M20	G37	B2 (Bacillus spp.)	B3 (Bacillus spp.)	B1 (Bacillus spp.)	mean
10	(63.08)53	(56.49)48	(47.05)43	(47.10)43	(47.51)44	(49.10)44	(48.98)44	(46.08)43	(43.09)41	(50.14)45	(51.41)46	(50.01)45
7.5	(55.05)48	(51.51)46	(53.04)47	(52.28)46	(49.31)44	(46.94)43	(49.02)44	(42.06)40	(44.72)42	(39.84)39	(35.36)36	(47.19)43
5	(55.93)48	(49.12)44	(52.99)47	(48.62)44	(47.29)43	(47.26)43	(42.37)40	(41.72)40	(40.59)40	(30.9)34	(42.12)40	(45.36)42
2.5	(49.41)44	(48.71)44	(42.73)41	(43.79)42	(42.56)41	(44.34)42	(35.37)36	(41.78)40	(40.19)39	(39.49)39	(29.90)33	(41.67)40
Mean	(55.87)48	(51.46)46	(48.95)44	(47.95)44	(46.67)43	(46.91)43	(43.94)41	(42.91)41	(42.15)40	(40.09)39	(39.70)39	
LSD for interaction			(±4.47)									
LSD for bacteria			(±2.23)									
LSD for concentration			(±1.34)									

( ) indicates arcsine transformed data.
[1]Aqueous
[2] Nutrient broth

Table 5: Effects of bacteria on *S. hermonthica* seeds germination in response to ACC

			Germination (%)					
Treatment	Conditioning medium		Bacteria					
ACC µM	A1	B2	*B. japonicum*	*P. putida*	*A. amazonas*	*Azotobacter*	*A. brasilense*	*A. brasilense*+ *P. putida*	Mean
10	(71.59)58	(68.96)56	(66.45)54	(60.48)51	(68.84)56	(60.61)51	(39.99)39	(35.12)36	(59.0)50
7.5	(65.69)54	(62.56)53	(58.14)50	(61.14)51	(65.19)54	(66.91)55	(40.99)40	(29.78)33	(56. 30)48
5	(64.55)54	(51.54)46	(54.57)48	(57.79)50	(64.18)53	(61.49)51	(38.13)38	(29.79)33	(52.76)47
2.5	(57.25)49	(55.43)48	(54.51)48	(54.41)47	(61.70)52	(54.32)47	(25.58)31	(28.62)33	(48.98)44
mean	(64.77)54	(59.62)51	(58.42)49	(58.46)50	(64.98)54	(60.83)51	(36.17)37	(30.83)34	
LSDfor interaction		(±4.95)							
LSD for bacteria		(±2.47)							
LSD for concentration		(±1.75)							

( ) indicates arcsine transformed data.
[1]Aqueous
[2]Nutrient broth

Table 6: Effects of combinations of bacteria on *S. hermonthica* seeds germination in response to ACC

			Germination (%)							
	Conditioning medium		Bacteria							
Treatment ACC µM	A1	B2	*P. putida + B. japonicum*	*Azotobacter+ B. japonicum*	*Azomonas+A. braslense*	*Azomonas+ B. japonicum*	*P. putida+ klebsiella*	*A. brasilense+ klebsillia*	*P. putida + Azomonas*	mean
10	(52.87)47	(43.89)42	(30.19)33	(37.87)38	(34.87)36	(36.44)37	(38.09)38	(40.90)40	(36.52)37	(39.0)39
7.5	(40.78)40	(36.96)37	(29.17)33	(29.89)33	(33.35)35	(43.38)41	(38.74)39	(37.40)37	(34.04)36	(35.97)37
5	(38.32)38	(36.3)37	(19.07)26	(34.34)36	(27.58)32	(36.55)37	(35.47)36	(39.06)39	(41.35)40	(34.23)36
2.5	(36.71)37	(34.85)36	(16.35)24	(21.57)28	(27.24)31	(29.80)33	(39.48)39	(24.85)30	(39.39)39	(30.03)33
Mean	(42.17)40	(38.00)38	(23.69)29	(30.92)34	(30.76)34	(36.54)37	(37.95)38	(35.55)37	(37.82)38	
LSD for interaction		(±7.11)								
LSD for bacteria		(±3.55)								
LSD for concentration		(±2.37)								

( ) indicates arcsine transformed data.
[1]Aqueous
[2] Nutrient broth

Table 7: Bacterial isolates, strains and combination suppressive to germination in *S. hermonthica*

GR24	ACC
Bacillus spp.(B1)	Bacillus spp.(B1)
Bacillus spp. (B2)	Bacillus spp. (B2)
-	Bacillus spp.(B3)
G14	-
Bradyrhizobium spp.	-
A. brasilense and Azomonas spp	-
-	A. brasilense
G8	-
M7	-
D50	-
G37	G37
M20	M20
D8	D8
#NAME?	-
Azomonas spp.+Bradyrhizobium	-
Azomonas spp.+ P. putida	-
Klebsiella sp.+ A. brasilense	-
P. putida+ Bradyrhizobium spp.	P. putida+ Bradyrhizobium spp.
P. putida+ Klebsiella sp.	-
-	Bradyrhizobium spp. and Azotobacter
-	A. brasilense and P. putida
-	A. brasilense and Azomonas

Effects of bacteria on haustorial initiation in S .hermonthica

Results showed that *Striga* germling resulting from seeds conditioned in water and /or nutrient broth medium showed similar response to DMBQ. However, haustorium initiation by the root macerate was significantly reduced. (Table 8). Four of the seven bacteria tested {B1 (*Bacillus* spp.), M2, G8C and G37} effected significant inhibition of haustorium induction by DMBQ in comparison to the corresponding nutrient broth control. For the root macerate only two isolates (M2, and G8C) significantly reduced haustorium initiation in comparison to the corresponding nutrient broth medium control.

Striga germlings resulting from seeds conditioned in water were more responsive to DMBQ than those conditioned in the nutrient broth medium. Of the eight bacteria tested only three (G7, *Azotobacter* and G8) affected significant inhibition of haustorium initiation in response to DMBQ in comparison to the corresponding nutrient broth control. The combination *Azotobacter* and *B. japonicum* had no effect (Table 9). In among the effective bacteria *Azotobacter* caused the highest inhibition, while G7 was the least. For the root macerate, five of the strains and isolates tested (*Klebsiella*, *P. Putida*, G14, G8 and *A. amazonas*) displayed no inhibitory effects on haustorium initiation. However, *Azotobacter* alone and in combination with *B. japonicum* effected significant inhibition (Table 9).

DMBQ and root macerate applied to *Striga* germlings resulting from seeds previously conditioned in water and treated with GR24 induced 49 and 40% haustoria, respectively (Table 10). Seed conditioned in nutrient broth medium, stimulated to germinate with GR24 and similarly treated with DMBQ and root macerate displayed 48 and 39% haustoria, respectively (Table 10). The effects of bacterial isolates on haustorial initiation varied from non- significant to significant when compared with the nutrient broth medium. Seven of the bacterial isolates screened had no effects on haustorium initiation. The isolate G18 caused a significant reduction in haustorial initiation, irrespective of the haustorium inducing factor. The isolate D32 had no effect on haustorium induction by DMBQ. However, haustorium initiation by the root macerate was significantly reduced. Isolate G11a, on the other hand, promoted haustorium induction significantly, irrespective of the haustorium inducing factor. Seeds conditioned in presence of the isolate, triggered to germinate with GR24, displayed 66 and 55% haustoria initiation in response to DMBQ and root macerate, respectively. (Table 10).

Table 8: Effects of bacteria on haustorium initiation in *S. hermonthica*

			Haustoria (%)							
	Conditioning medium		Bacteria							
Treatment	A1	B2	B1(*Bacillus* spp.)	M2	G8C	G37	G18a	D10	M20	Mean
DMBQ	(54.45)47	(54.76)48	(44.21)42	(41.96)40	(45.57)42	(45.00)42	(50.21)45	(53.22)47	(53.11)47	(49.16)44
root macerate	(51.91)46	(42.53)41	(33.87)36	(26.60)31	(28.12)32	(41.71)40	(43.07)41	(46.30)43	(35.96)37	(38.90)39
Mean	(53.18)47	(48.64)44	(39.04)39	(34.28)37	(36.84)37	(43.35)41	(46.64)43	(49.76)45	(44.53)42	
LSD for interaction		(±8.72)								
LSD for bacteria		(±6.17)								
LSD for inducing factors		(±2.90)								

( ) indicates arcsine transformed data
[1]Aqueous
[2] Nutrient broth

Table 9: Effects of different bacteria on haustorium initiation in *S. hermonthica*

			Haustoria (%)								
	Conditioning medium		Bacteria								
Treatment	A[1]	B[2]	G7	*Azotobacter*	G8	*P.putida*	G14	A. *amazonas*	*klebsiella*	*B. japonicum +Azotobcter*	mean
DMBQ	(54.78)48	(43.50)41	(31.18)34	(29.10)32	(28.06)33	(32.07)34	(39.96)39	(45.0)42	(47.41)43	(36.09)37	(38.71)38
root macerate	(42.21)40	(36.71)37	(26.65)31	(22.37)28	(45)42	(26.29)31	(33.91)36	(33.85)36	(26.86)31	(22.31)28	(31.62)34
Mean	(48.49)44	(40.10)39	(28.91)33	(25.74)30	(36.53)37	(29.18)33	(36.93)37	(39.43)39	(37.14)37	(29.20)33	
LSDfor interaction			(±11.85)								
LSD for bacteria			(±8.38)								
LSD for inducing factors			(±3.74)								

( ) indicates arcsine transformed data.
[1]Aqueous
[2] Nutrient broth

Table 10: Effects of bacteria on haustorium initiation in *S. hermonthica*

			Haustoria (%)											
	Conditioning medium		Bacterial isolate											
Treatment	A1	B2	G18	D32	G11a	G6C	D46	D8	G7a	D49	G14	D20	M7	mean
DMBQ	(56.71)49	(55.36)48	(16.43)24	(47.11)43	(82.96)66	(52.6)46	(42.96)41	(48.44)44	(42.76)41	(50.91)46	(53.50)47	(45.72)43	(52.33)46	(49.84)45
Root macerate	(42.07)40	(39.85)39	(17.59)25	(21.27)27	(67.5)55	(43.1)41	(29.53)33	(35.11)36	(33.72)35	(41.74)40	(30.82)34	(34.02)36	(35.99)37	(36.34)37
Mean	(49.39)44	(47.61)44	(17.01)24	(34.19)36	(75.23)60	(47.9)44	(36.2437	(41.77)40	(38.24)38	(46.33)43	(42.16)40	(39.87)39	(44.16)42	
LSD for interaction		(±8.65)												
LSD for bacteria		(±6.11)												
LSD for inducing factors		(±2.40)												

( ) indicates arcsine transformed data.
[1]Aqueous
[2] Nutrient broth

Table 11: Bacterial isolates, strains and combination suppressive to haustorial initiation in *S .hermonthica*

Root macerate	DMBQ
G8C	G8C
M2	M2
G7	G7
P. putida	*P. putida*
-	G8
-	G37
Azotobacter	*Azotobacter*
Klebsiella spp.	-
Bradyrhizobium and *Azotobacter*	*Bradyrhizobium* and *Azotobacter*
Bacillus spp.(B1)	*Bacillus* spp.(B1)
M20	-
G18	G18
D32	D32
G14	-
G11a*	G11a*

* Stimulation

Discussion

Referring to the available published literature, this study provides the first detailed investigation on the possible use of soil borne bacteria for the control of *S. hermonthica* through inhibition and/or perturbation of the early developmental events in the parasite life cycle. This study demonstrates that a number of bacterial isolates and strains have the potential to reduce damage by *S. hermonthica*. The results revealed that some bacterial strains and isolates inhibited germination; some had no effects while other enhanced it (Tables 1-7), some bacterial strains and isolates had no effect on germination at high stimulant concentration, but they were inhibitory at low concentrations, haustorium initiation in response to DMBQ and sorghum root macerate were differentially influenced by bacterial strains and isolates.

The present study revealed that of the 211 bacterial strains, isolates and combination screened 11inhibited germination in response to GR24 but not ACC. Some inhibited ACC elicited germination, while others (6) inhibited germination in response to both (Tables 1-7). These observations may indicate that some bacteria may inhibit or reduce activity of ACC synthase without influencing ACC conversion into ethylene; some may promote ACC catabolism and/or conversion into ethylene. Others may reduce ethylene biosynthesis by influencing both ACC synthesis and oxidation. Another possibility could be that extremely high concentrations of exogenous ethylene might partially retard germination. Several soil microorganisms were reported to proliferate when ACC was supplied as the sole nitrogen source. However, no soil microorganism was reported to produce ethylene directly from ACC (Frankenberger and Arshad, 1995).

The present investigation revealed that haustorium initiation in response to DMBQ, and sorghum root macerate is inhibited by some of the bacterial strains and isolates (Tables 8-11). Moreover, the inhibitory effects showed dependence on the bacterium used and the source of the haustorium factors. This inhibition may be attributed to phytotoxic substances, inhibitors or extracelluar enzymes that degrade and/or curtail release of the haustorium factor from the host root. The differential inhibitory effects of the bacteria associated with the source of the haustorium inducer may be attributed to differential concentrations of inhibitors produced by the bacterium and/or concentration of the haustorium initiation factor in the root macerates. The root macerate may also contain various chemicals that inhibit or promote bacterial growth and/or production of haustorium inhibitors by the bacterium. Auxin and auxin-like compounds have been reported to inhibit haustorium initiation in *Striga* (Keyes *et al,.* 2000; Mabrouk *et al*., 2006). *Azotobacter* spp., *P. putida*, *A. brasilense* and *Klebsiella* spp. are known to produce auxin and auxin – like compounds in plants rhizosphere (Frankenberger and Arshad, 1995). Production of the haustorium factor (DMBQ) by intact sorghum roots requires production and release of H_2O_2 from the parasite root tip (Keyes *et al*., 2000). H_2O_2 is critical for activation of host perioxidases and oxidative release of DMBQ from the host epidermal cells (Keyes *et al*., 2000). Differential production of the enzyme catalase, which disproportionate H_2O_2 to H_2O and molecular oxygen, by bacterial isolates would lead to differential production of DMBQ and hence differential reduction in haustorium initiation (Key *et al*. 2000).

The potential of bacterial strains with capacity to inhibit germination of the parasite seeds, in general, deserve further research on isolation of more effective bacterial strains and expanded evaluation of the isolates identified in the present study.

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