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IN VITRO EVALUATION OF SOME SOIL RHIZOSPHERE BACTERIA FOR BIOLOGICAL CONTROL OF *STRIGA HERMONTHICA* (DEL.) BENTH

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Abstract: *Striga hermonthica* is a devastating root parasitic weed on cereals. In the present investigation a series of laboratory experiments was undertaken to study the effects of some soil borne bacteria on germination and haustorium initiation in *S. hermonthica*. A total of 211 bacterial strains and isolates were screened, in a series of preliminary laboratory experiments, for ability to reduce GR24-induced *Striga* germination. Isolates and strains displaying inhibitory effects (26) were further screened to confirm their suppressive effects on GR24-induced germination, study their influence on *Striga* germination in response to ACC and on haustorium initiation in response to DMBQ and sorghum root macerate. *Striga* seeds conditioned in a broth medium inoculated with bacteria showed differential response to GR24 and ACC. Some of the bacterial isolates and strains reduced germination, irrespective of the stimulant used, while others inhibited germination induced by one of the stimulants, but not the other. Haustorium initiation in response to DMBQ and sorghum root macerate showed differential response to bacterial strains and isolates. Some bacterial isolates and strains had no effects on haustorium initiation, while others reduced it, irrespective of the inducing factor. The differential response in germination to GR24 and ACC could be due to bacterial metabolites that curtail production of ACC or its conversion into ethylene. Curtailment of haustorium initiation by bacteria may be attributed to production of inhibitory compounds and/or extracellular enzymes that degrade DMBQ or curtail its production. The study supports the biotic nature of the reported soil suppressiveness to *S. hermonthica* and indicates that inoculation of soil with bacteria may perturb early developmental stages of parasitism and reduce the devastating effects of the parasite.

Key words: *Striga hermonthica*, Suppression, Rhizosphere bacteria, Biocontrol**.**

INTRODUCTION

Sorghum [*Sorghum biocolor* (L.) Moench], a Poaceae, is an important cereal in Africa. Its production is constrained by both biotic and a biotic factors. It can be infested by many above and below – ground pests and pathogens including bacteria, fungi, viruses and weeds (Lendzemo, 2004). Parasitic plant species of the genus *Striga* are obligate root parasites that constitute a major constraint to cereal and legume production in the savannas of Africa (Emechebe et al., 1991). The life cycle of *Striga* spp. is modulated by exchange of signals between the host, the biotic environment and the parasite (Ransom and Njorage, 1991). To germinate *Striga* species need an after-ripening period, pre-treatment in warm moist conditions (conditioning) and subsequently exposure to a germination stimulant. Following to germination, a haustorium, organ of attachment and a physiological bridge between the host and the

parasite, is formed on perception of a second host derived signal (Riopel et al., 1990).

The physiological mechanisms involved in the conditioning process were attributed to an increase in permeability of cellular membranes, leaching of an inhibitor and/or promotion of synthesis of a germination stimulant (Worsham, 1987). 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 seeds (Babiker et al., 2000). Germination stimulants, natural or synthetic, induce ethylene biosynthesis in *Striga* seeds. Haustorium initiation, which represents the switch from the vegetative to the parasitic mode of life, is induced by 2, 6-dimehoxy-p-benoquinone (DMBQ). DMBQ synthesis involves activation of peroxidases in host root by hydrogen peroxide generated in the radicle of *Striga* germlings. The haustorium, attaches, penetrates the host and establishes connection with host xylem. *Striga* seedlings develop and remain subterranean for 6 to 8 weeks, during which time the parasite is entirely dependent on the host and is most damaging. Curtailment of germination and/or haustorium initiation reduces parasitism and consequently host damage by the parasite.

Biological control of weeds with insects and microbial agents utilizes of living organisms to manipulate, suppress, reduce, or eradicate weeds (Traore et al., 1996). The use of rhizobacteria for biological control of *Striga* is intriguing since they can easily be formulated as seed inoculants, thereby avoiding the need for application equipment, voluminous carriers and labour that would, otherwise, be cost prohibitive (Miche et al., 2000). Recently, several bacterial isolates and strains were reported to suppress germination of *S. hermonthica* (Miche et al., 2000, Ahonsi et al., 2002). In addition (Ahonsi et al., 2002) screened 460 *Pseudomonas fluorescens* isolates from naturally suppressive soils and identified 15 isolates of which *P. fluorescens* and *P. putida* caused significant inhibition of *S. hermonthica* germination. These reports may provide a novel approach for combating the parasite. 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 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

S. hermonthica seeds were collected in 2004 from infected sorghum fields at the Gezira Research Station Farm, Sudan. Seeds were surface sterilized as described by (Hsiao *et al*., 1981). Briefly, the seeds were soaked in 70 % for 2 minute in ethanol and rinsed three times with distilled water. Subsequently the seeds were immersed in 1% NaOCl solution for 3 minutes with continuous agitation, thoroughly washed with sterilized distilled water; air dried and kept in sterilized vials, at ambient temperature till used.

Chemicals

Striga germination stimulant GR24 was 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 a gift from Dr. Sugimoto, Y. from Koby University, Japan.

Sorghum root macerate preparation

Seeds of sorghum cv. Tabat (*Striga* susceptible) were surface disinfected by immersion in an aqueous solution of 1 % sodium hypochlorite, commercial Bleach, for 20 minute. The seeds, thoroughly washed with sterilized distilled water were planted in sand, in plastic pots (12 cm i.d.), perforated at the bottom. Roots, harvested 21 days after sowing, were thoroughly washed with distilled sterilized water. Root samples (1 g each) were crushed in 10 ml of sterilized distilled water in a mortar. 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

Soil samples were collected from four locations namely Shambat, Gadaref, Abuharaz and Wad Medani. The samples were obtained from the top 5-10 cm soil layer in 20 sorghum fields in each site. A general purpose medium [Nutrient Agar (2.8%)] was used for culturing of bacterial isolates. Two hundred and two bacterial isolates were obtained from the soil samples. In

addition, six bacteria strains *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 glass fiber filter papers (GF/C) discs (8 mm diameter) were cut, wetted thoroughly with water and placed in an oven at 100 ºC for 1 h to be sterilized and ready for further use. The 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 bacterium isolate or strains. About 25-50 surface disinfected *S. hermonthica* seeds were sprinkled on each of the glass fiber discs in each petri dish. The dishes, sealed with parafilm were placed in black polythene bags and incubated at 30 ºC in the dark for 10 days.

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

A total of 211 isolates and strains 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 selected strains, isolates and a combination of *A. amazonas* plus *P. putida*. The preliminary experiment was conducted in two batches, each batch was screened, under the same conditions on a separate day. Each experimental run included two controls comprising *S. hermonthica* seeds conditioned in sterile distilled water and seeds conditioned in diluted nutrient broth medium. In the confirmatory screening, the 26 bacterial isolates, strains and the combination of *A. amazonas* plus *P. putida*, selected on basis of the results of the preliminary screening, were re-screened using the previously described procedure. In both the preliminary and confirmatory screening *Striga* seeds were treated with GR24 at o. oo , 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*

Twenty two bacterial strains, isolates and a combination of *A. amazonas* plus *P. putida* were screened for their ability to inhibit ACC-induced germination of *S. hermonthica* seeds. Conditioned seeds were treated with aliquots (20µl) of ACC at 2.5, 5, 7.5 and 10 µM or distilled water, as previously described. The seeds were reincubated and examined for germination.

Effects of bacteria on haustorial initiation in *S. hermonthica*

In this experiment seventeen bacterial strains and isolates were screened for their effects on haustrorium initiation. *Striga* seeds conditioned in presence and absence of bacterial isolates and/or strains as described above, were treated, each, with 20 µl GR24 solution (0.34 µM) were incubated in the dark at 30 ºC for 48 h. 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. The Petri dishes were incubated in the dark at 30 ºC for an additional 24 h and then examined for haustorium initiation using a binocular stereomicroscope.

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 compared with the least significance difference (LSD) at 5% level. The data were back transformed and tabulated.

RESULTS

Laboratory experiments

Effects of bacterial isolates and strains on GR24 -induced germination of *S. hermothica* seeds

Results from the preliminary screening revealed that few isolates and strains enhanced germination; some had no effects, while others showed significant suppression. Confirmatory screening employing bacterial isolates and strains showed that seeds treated with distilled water displayed negligible germination in all

experiments, GR24 at 0.034 – 3.4 µM effectively induced germination of water conditioned seed in a dose dependent – manner and a number of bacterial isolates and strains reduced germination significantly in comparison with the control (Tables 1-2).

GR24 applied to seed conditioned in water induced the highest germination (54 - 57 %) (Table 1). Conditioning in the nutrient broth medium reduced germination to between 46 and 52 %. The depressive effects of the medium decreased with increasing GR24 concentration. All bacterial isolates and strains, except *A. amazonas* and *Azomonas* spp. decreased *S. hermonthica* germination in response to GR24 in comparison with the corresponding aqueous and nutrient broth medium controls. *A. amazonas* had no effect on germination at the higher concentrations of GR24 (0.34 and 3.4 µM). However, at the lowest concentration (0.034 µM), the bacterium reduced germination significantly. A combination between *A. amazonas* and *P. putida* was more suppressive to *S. hermonthica* germination than *A. amazonas,* alone, but germination was comparable to that attained with *P. putida*. In among all bacterial isolates D49 and D46 were, consistently, the inhibitoriest.

GR24 applied to seed conditioned in water exhibited 53 - 55% germination. Seed conditioned in the nutrient broth and similarly treated with GR24 displayed comparable germination (Table 2). The suppressive effects of the isolates on *Striga* seeds germination varied from non- significant to highly significant when compared with the nutrient broth. In among the isolates tested, only GSL and S22 effected significant reductions in germination (Table 2).

Effects of bacterial strains and isolates on ACC - induced germination of *S. hermonthica*

Seeds treated with distilled water displayed negligible germination in all experiments (Tables 3– 5). ACC at 2.5, 5, 7.5 and 10 µM effectively induced germination of conditioned seeds in a dose- dependent manner. *Striga* seeds exhibited high germination (51 - 59%) in response to a terminal ACC treatment, irrespective of the conditioning medium (Table 3). All bacterial isolates tested, except D10 and G18x showed no adverse effects on germination. Isolate D10 had inconsistent effects. However, isolate G18x reduced germination, significantly, at all ACC concentrations.

ACC applied to seeds conditioned in water induced 47- 57 % germination (Table 4). Conditioning in the nutrient broth reduced germination in response to ACC to between 45 and 54 %. The depressive effects of the medium decreased with increasing ACC concentrations. All bacterial isolates tested, except S25 and D25, showed inconsistent performance that varied from no effect, to slight inhibition or slight stimulation. Isolates S25 and D25 on the other hand, consistently affected significantly lower germination than the corresponding nutrient broth control.

Effects of bacterial isolates and strains on haustorial initiation in *S. hermonthica*

S. hermonthica germlings resulting from seeds conditioned in water or nutrient broth medium showed similar response to DMBQ and root macerate (42 - 39 % haustorium initiation) (Table 6). Seven of the bacterial isolates tested had no effect on haustorial initiation in response to DMBQ, while three of the isolates (D2, G11, and GSL) caused significant reduction. In among the three isolates the inhibitory effect was highest for GSL (69%) and lowest for D2 (19%) in comparison to the nutrient broth medium control. The response to root macerate was different. All isolates effected significant inhibition of haustorium initiation. The isolates G18x, S23, S19, G6C and M34 reduced haustorium initiation to between 31 and 74% of that attained with the nutrient broth control.

Striga germlings, resulting from seeds conditioned in water or broth medium showed more or less similar response to DMBQ and root macerate (Table 7). Of all the bacteria tested five showed no significant reduction in haustorium initiation in response to DMBQ. However, two isolates D25ok and S9 reduced haustorium initiation significantly in comparison to the corresponding nutrient broth control. For root macerate only *Bradyrhizobium* spp. caused a considerable inhibition of haustorium initiation, other isolates were, however, not effective (Table 7).

Table 1. Effects of bacteria on GR24-induced *S. hermonthica* seed germination

	Germination (%)													
	Bacterial isolates/ strains													
GR24 (µM)	**Conditioning medium** A[1]	B[2]	D49	D46	*P.putida*	M2	*A. braslense*	D10	G18x	*A.amazons*	*Azomonas*	*A.amazons +P.putida*	GSC	mean
3.4	(70.98) 57	(63.10) 52	(36.84) 37	(42.08) 40	(46.14) 43	(60.96) 51	(45.15) 42	(46.25) 42	(48.93) 44	(61.08) 51	(61.70) 52	(41.88) 40	(46.80) 43	(51.74) 46
0.34	(65.89) 54	(57.63) 49	(27.52) 32	(36.00) 37	(45.38) 42	(50.95) 46	(32.47) 35	(40.82) 40	48.25) 44	(58.85) 50	(63.78) 53	(42.00) 40	(46.14) 43	(47.40) 43
0.034	(66.50) 55	(52.04) 46	(15.65) 23	(25.70) 30	(31.99) 34	(40.67) 40	(30.12) 33	(38.90) 39	(19.60) 26	(43.24) 41	(55.47) 48	(35.42) 36	(39.59) 39	(38.07) 38
mean	(67.79)a 55	(57.59) 49	(26.67) 31	(34.59) 36	(41.17) 40	(50.86) 45	(35.91) 37	(41.99) 40	(38.92) 39	(54.39) 47	(60.32) 51	(39.76) 39	(44.18) 42	

LSD for interaction (±6.02) LSD for bacteria (±3.47) LSD for concentration (±1.66)

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

Table 2. Effects of bacteria on GR24-induced *S. hermonthica* seed germination

	Germination (%)																	
	Conditioning medium		**Bacterial isolates**															
GR24 (µM)	A[1]	B[2]	GSL	S22	S19	D25	G18x	M34	S25	S10	G11	S23	G4C	D2a	G6C	S9	G11a	mean
3.4	(67.07) 55	(65.24) 54	(46.93) 43	(46.81) 43	(53.94) 47	(55.77) 48	(58.36) 50	(59.25) 50	(51.47) 46	(65.61) 54	(48.68) 44	(52.11) 46	(61.83) 52	(55.97) 48	63.08) 53	(60.32) 51	(57.12) 49	(57.03) 49
0.34	(66.43) 54	(64.42) 53	(48.68) 44	(45.10) 42	(57.23) 49	(52.39) 46	(64.65) 53	(65.43) 54	(60.05) 51	(60.99) 51	(52.44) 46	(60.29) 51	(59.17) 50	(52.26) 46	(51.85) 46	(57.86) 49	(58.44) 50	(57.51) 49
0.034	(63.78) 53	(63.05) 53	(41.76) 40	(39.14) 39	(55.33) 48	(49.41) 44	(53.19) 47	(52.47) 46	(54.87) 48	(64.16) 53	(51.78) 46	(58.00) 50	(56.24) 48	(49.86) 45	(46.94) 43	(55.05) 48	(57.88) 49	(53.70) 47
mean	(65.76) 54	(64.24) 53	(45.79) 43	(43.69) 41	(55.50) 48	(52.53) 46	(58.73) 50	(59.05) 50	(55.46) 48	(63.59) 53	(50.96) 46	(56.80) 49	(59.08) 50	(52.70) 47	(53.96) 47	(57.74) 49	(57.81) 49	

LSD for interaction (±6.20) LSD for bacteria (±3.58) LSD for concentration (±1.50)

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

Table 3. Effects of bacteria on *S. hermonthica* seeds germination in response to ACC

Treatment ACC µM	Conditioning medium A[1]	Conditioning medium B[2]	Germination (%) Bacterial isolate/ strains G18x	D10	M2	D49	*A. amazonas+ P. putida*	*Azomonas*	Gsc	*klebsiella*	mean
10	(72.74) 59	(70.06) 57	(62.58) 52	(64.10) 53	(66.08) 54	(68.23) 56	(65.13) 54	(70.26) 57	(68.49) 56	(71.16) 57	(68.20) 56
7.5	(72.30) 58	(66.59) 55	(57.32) 49	(59.04) 50	(63.50) 53	(66.11) 54	(65.01) 54	(70.10) 57	(65.95) 54	(69.10) 56	(66.64) 55
5	(72.48) 58	(62.14) 52	(53.99) 47	(63.19) 53	(63.31) 53	(63.94) 53	(62.41) 52	(69.74) 57	(64.71) 54	(67.23) 55	(65.59) 54
2.5	(67.71) 56	(60.36) 51	(52.55) 46	(56.74) 49	(62.96) 52	(59.96) 51	(58.05) 50	(66.58) 55	(62.96) 52	(67.44) 55	(62.70) 52
mean	(71.31) 57	(64.79) 54	(56.61) 49	(60.77) 51	(63.96) 53	(64.56) 53	(62.66) 52	(69.17) 56	(65.53) 54	(68.73) 56	

LSD for interaction (±5.02) LSD for bacteria (±2.51) LSD for concentration(±1.58)

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

[1]Aqueous [2]Nutrient broth

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

Treatment ACC µM	Conditioning medium A[1]	Conditioning medium B[2]	Germination (%) Bacterial isolate S25	D25	G6c	D2	S9	S22	S10	G14	D20	G11a	M34	S23	G4c	S19	GSL	mean
10	(71.31) 57	(66.41) 54	(50.78) 46	(58.61) 50	(59.88) 51	(65.29) 54	(60.42) 51	(66.01) 54	(60.42) 51	(62.65) 53	(58.86) 50	(64.46) 53	(64.58) 54	(58.26) 50	(61.11) 51	(63.50) 53	(65.61) 54	(62.24) 52
7.5	(68.23) 56	(60.53) 51	(51.82) 46	(54.47) 47	(58.14) 50	(62.12) 52	(61.84) 52	(65.29) 54	(51.41) 46	(59.37) 50	(60.21) 51	(68.32) 56	(63.02) 53	(59.93) 51	(61.52) 52	(65.48) 54	(62.74) 53	(60.85) 51
5	(64.24) 53	(53.60) 47	(46.08) 43	(45.52) 43	(53.12) 47	(61.07) 51	(59.77) 51	(61.89) 52	(57.62) 50	(56.13) 48	(51.68) 46	(65.97) 54	(63.37) 53	(56.28) 48	(60.89) 51	(61.86) 52	(60.05) 51	(57.60) 50
2.5	(53.98) 47	(50.02) 45	(41.17) 40	(41.55) 40	(40.94) 40	(50.28) 45	(52.41) 46	(59.44) 50	(49.24) 44	(52.82) 47	46.26) 43	61.51) 52	(51.32) 46	(45.04) 42	(51.23) 46	(49.20) 44	(45.83) 43	(49.54) 45
mean	(64.44) 53	(57.64) 50	(47.46) 43	(50.04) 45	(53.02) 47	(59.69) 51	(58.61) 50	(63.16) 53	(54.67) 48	(57.74) 49	(54.25) 47	(65.06)54	(60.57)51	(54.88)48	(58.69)50	(60.01) 51	(58.56) 50	

LSD for interaction (±4.39) LSD for bacteria (±2.19) LSD for concentration (±1.06)

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

Table 5. Bacterial strains and isolates suppressive to germination in *S. hermonthica*

GR24	**ACC**
D49	-
D46	-
S22	-
GSL	-
M2	-
P. putida	-
P. putida and A.amazonas	-
-	S25
-	D25
S23	-
D10	D10
G18x	G18x

Table 6. Effects of bacteria on haustorium initiation in *S. hermonthica*

	Haustoria (%)												
	Conditioning medium		**Bacterial isolate**										
Treatment	A[1]	B[2]	S22	S19	G18x	G6C	S23	S10	M34	D2	G11	GSL	mean
DMBQ	(45.32) 42	(44.59) 42	(40.68) 40	(41.33) 40	(43.24) 41	(38.90) 39	(43.51) 41	(37.73) 38	(37.24) 37	(32.04) 34	(29.85) 33	(4.96) 13	(36.62) 37
Root macerate	(40.33) 39	(39.83) 39	(29.28) 33	(18.07) 25	(20.74) 27	(20.04) 27	(19.92) 26	(29.81) 33	(3.15) 10	(26.30) 31	(23.99) 29	(20.77) 27	(24.35) 29
Mean	(42.83) 41	(42.21) 40	(34.98) 36	(29.70) 33	(31.99) 34	(29.47) 33	(31.72) 34	(33.77) 35	(20.19) 27	(29.17) 33	(26.92) 32	(12.86) 21	
	LSD for interaction (±9.20)				LSD for bacteria (±6.51)			LSD for inducing factors (±2.56)					

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

Table 7. Effects of bacteria on haustorium initiation in *S. hermonthica*

Treatment	Haustoria (%)									
	Conditioning medium		Bacterial isolates/ strain							
	A[1]	B[2]	S9	D25ok	*B. japonicum*	S25	D25	GSC	G4c	mean
DMBQ	(53.41)	(51.36)	(27.32)	(22.5)	(38.09)	(50.27)	(41.53)	(47.76)	(50.62)	(42.54)
	47	46	31	28	38	45	40	44	45	41
root	(51.63)	(47.44)	(38.14)	(51.54)	(25.13)	(45.51)	(34.22)	(50.31)	(51.77)	(44.12)
macerate	46	43	38	46	30	42	36	45	46	42
Mean	(52.92)	(49.40)	(32.73)	(37.02)	(31.61)	(47.89)	(37.87)	(49.04)	(51.19)	
	47	44	35	37	34	44	38	44	46	

LSD for interaction (±23.36) LSD for bacteria (±16.52) LSD for inducing factors (±7.78)

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

Table 8. Bacterial strains and isolates suppressive to haustorial initiation in *S. hermonthica*

DMBQ	Root macerate
-	G6C
D2	D2
G11	G11
GSL	GSL
S9	-
D25ok	-
-	G18x
-	S23
-	S19
-	S10
-	M34
-	S22
-	*B. japonicum*

DISCUSSION

The present investigation, demonstrated the potentials of native soil borne bacteria to perturb early developmental stages of *S. hermonthica*. Some bacterial strains and isolates inhibited germination; some had no effects while others enhanced it (Tables 1- 4). 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 was differentially influenced by bacterial strains and isolates.

Several of the bacteria isolates (26 out of 211) suppressed *Striga* seed germination. However, of these bacterial isolates only few (D49, D46, *A. brasilense* and *P. putida*) effected consistent and highly significant inhibition (Table 1 - 5). These findings are consistent with the suggestion made by Berner et al., (1996) that curtailment of *Striga* parasitism in suppressive soil could be attributed to microbial action. Both inhibition and promotion of *Striga* germination can be achieved by manipulation of ethylene biosynthesis, ethylene action, or by promotion of ethylene metabolism or that of its immediate precursor ACC. Ethylene biosynthesis in plants is regulated by two key enzymes ACC synthase and ACC oxidase (Babiker et al*.,* 1993, Babiker et al., 2000). Aminoethoxyvinyl glycine (AVG), an ACC synthase inhibitor, curtails ethylene biosynthesis and germination in *Striga (*Babiker et al., 1993*)*. Norbornadiene, silver thiosulphate and high concentrations of CO_2, inhibitors of ethylene action, also inhibit *Striga* germination (Babiker et al., 2000, Parker and Riches 1993, Imaseki 1991). In plants, ACC is deactivated by malonylation to N- malonyl ACC (MACC), while it is deactivated by microorganisms by conversion to α- aminobutyric acid and ammonia (Imaseki 1991).

The inhibitory effects of the bacterial strains and isolates applied during conditioning could be attributed to a direct effect of the isolate on the seed or indirectly through production of chemical(s) that is/are toxic to the seeds, inhibitors of ethylene biosynthesis, inhibitors of ethylene action, promoters of ethylene deactivation and/or promoters of ethylene biosynthesis (Imaseki 1991, Babiker et al., 1993, Babiker 2007, Hassan et al.; 2009). Germination stimulants, including ethylene applied during conditioning of *Striga* seeds were reported to reduce germination (Hsiao et al., 1981).

Haustorium initiation in response to DMBQ and sorghum root macerate is inhibited by some of the bacterial strains and isolates (Tables 6 - 8). Moreover, the inhibitory effects showed dependence on the bacterium used and the source of the haustorium factor. *B. japonicum* and isolates D2, G11, GSL D25ok, and M34 inhibited haustorium initiation in response to both. D25ok and S9 reduced haustorium initiation in response to DMBQ, while G18x, S23, S19, G6C, S22 and D25 were inhibitory to haustorium initiation in response to root macerate. Inhibition of haustorium initiation in *Striga* by bacteria is in line with numerous reports (Hassan et al., 2008, Mabrouk et al*.,* 2006, Keyes et al., 2000) and may be attributed to phytotoxic substances, inhibitors or extracelluar enzymes that degrade, curtail release of the haustorium factor from the host root, degrade and/or reduce production of H_2O_2 in *Striga* radicle tip. 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). Auxin and auxin-like compounds have been reported to inhibit haustorium initiation in *Striga* (Keys et al., 2000). *P. putida*, *A. brasilense* and *Klebsiella* spp. are known to produce auxin and auxin – like compounds in plants rhizosphere (Frankenberger and Arshad, 1995).

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.

This study supports the biotic nature of soil suppressivneness to *S. hermonthica* as suggested by Berner et al., (1996). Moreover, it indicates that inoculation of soil with bacteria may perturb the early developmental stages of the parasite and reduce its deleterious effects on the hosts. Circumstantial evidence suggests the possible involvement of phytohormones in bacterial suppression of *Striga* germination and haustorium initiation. Future research should focus on re-screening of the effective bacteria and bacterial isolates and rank them according to their ability to suppress or promote specific stages in *Striga* life cycle. In an endeavour to

work out possible bacterial combinations that effect more than one developmental stage in the parasite life cycle. Furthermore, factors affecting activity of the bacterial isolates and strains should be determined to ensure maximum suppressive effects. An important point to note is that the culture medium may influence germination and subsequent development of the parasite. Proper dilutions have to be made and an un-inoculated broth should be included as a control.

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