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Full Length Research Paper

Evaluation of some bacterial isolates as germination stimulants of *Striga hermonthica*

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To investigate the role of *Pseudomonas* sp., *Klebsiella oxytoca* and *Enterobacter sakazakii* in *Striga hermonthica* seed germination, we have used two varieties of sorghum to test over time for the selected bacteria, using GR-24, a synthetic strigol analogue as a standard and water as a check. Sorghum seeds coated with a mixture of bacterial cells (5 x 10^7 cfu ml^{-1}) from 24 h old cultures were planted in pasteurized potted soil, which was infested with 0.05 g (about 3000) viable *S. hermonthica* seed per pot 14 d before sowing. Results have indicated that the bacterial isolates could stimulate *S. hermonthica* germination in the laboratory and in the screen-house. In vitro, only *Pseudomonas* sp. 4MKS8 gave significant stimulation of *S. hermonthica* seed at 5% level of probability as compared to the water check. In the screen-house, the four bacterial isolates stimulated significant germination of *S. hermonthica*.

Key words: *Striga hermonthica, Pseudomonas* sp., *Klebsiella oxytoca, Enterobacter sakazakii*, germination stimulant, sorghum.

INTRODUCTION

In the sub-Saharan Africa, it is a common occurrence for the plants to be attacked by one pathogen or another. One of such pathogens attacking plant is *Striga*, a genus of obligate root-parasitic flowering plant belonging to the family Scrophulariaceae. *Striga* species constitutes a big constraint to sustainable development and poverty alleviation. The main graminaceous crops attacked by *Striga hermonthica* include maize, sorghum, pearl millet, finger millet, upland rice and sugar cane (Berner et al., 1995). In addition to the conditions necessary for the germination of any seed, *Striga* requires the presence of a stimulant.

Today many farmers lack an understanding of its biology and parasitic nature and so cannot manage *Striga* in the field. Researchers have come to understand that no single methodology will be completely effective in eliminating *Striga* infestations in farmer's fields. Meanwhile research efforts by scientists employ multidisciplinary approach in the combat of *Striga*. Microorganisms are increasingly being considered as control agents for *Striga* (Babalola et al., 2002; Babalola et al., 2004) . Among the micro- organisms colonising the root surface are bacteria of the genus *Pseudomonas* (Babalola, 2002) of which fluorescent pseudomonads are of special importance (Vancura, 1980). The composition of the microbial association on the root surface can be modified by the introduction of bacterial cells on the surface of seed, roots or tubers. Plant growth-promoting rhizosphere bacteria possess a number of physiological properties, which are important to colonisation of the surface of plant roots and enhancing the growth and health of plants (Vancura and Kunc, 1987). Several methods are available to control *Striga* including cultural practices, application of fertilizers, herbicides, and germination stimulants, and planting resistant crop varieties. However, none of these methods alone provides acceptable levels of control (Abbasher et al., 1996) . Therefore, the objective of this study was to increase suicidal germination of *Striga* by using bacteria inoculants in mixed cropping of cowpea with sorghum.

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MATERIALS AND METHODS

Isolation of rhizosphere bacteria

Root samples of potted maize and sorghum were shaken vigorously by hand to remove loosely adhering soil. Bacteria from the region of soil neighbouring the root and influenced by it (exorhizosphere, Klyuchnikov and Kozhevin, 1991) and intercellular spaces accessible to bacteria (endorhizosphere) were obtained from 1 g firmly adhering soil or macerated roots (Mawdsley and Burns, 1994). 1 g of firmly adhering soil was shaken in 100 ml of 0.01 M phosphate buffer solution (PBS) solution (0.88% (w/v) NaCl, 2.9 mM KH_2PO_4, 7.1 mM K_2HPO_4, pH 7.2) for 2 h. on a rotary shaker (Orbit shaker bath, U.S. Patent No: Des. 288.600, U.S.A) at 200 rpm. Surface sterilisation effected by immersion in 70% ethanol for 30 s. and in 4% solution of sodium hypochlorite (NaOCl) for 3 min. was carried out on the root sample to ensure that the isolates to be cultured were from the endorhizosphere. These were transferred to the laminar flow hood (ENVIRCO, Environmental Air Control, Inc.). Subsequently, serial dilutions (up to 10^{-8}) were prepared, beginning with a 1 ml aliquot of the stock suspension. One ml of the suspensions from the dilution levels 10^{-3} to 10^{-8} was dispensed in separate 9 cm diameter sterilised Petri-dishes containing King's medium B (KB) in triplicate. The plates were swirled gently in a clockwise motion to mix the suspension over the agar. After incubation for 24 h at 28^{O}C, representative types of bacterial colonies were further purified on KB and stored in 35% glycerol at -80^{O}C. One hundred and eighty isolates of rhizosphere bacteria were screened in preliminary laboratory tests for *in vitro* stimulation of *Striga. hermonthica*. Four potential isolates, (isolates in which *S. hermonthica* germination was observed) were further tested in the screen-house by seed coating.

S. hermonthica germination test

Striga seed viability was carried out according to the method of Eplee and Norris, (1987). Variability in infection, which may be due to differences in age and quality of inoculum, hosts, growth conditions, and levels of infestation was minimised by using the same seed stock of *S. hermonthica,* Bida 97. These seed were collected from the same site (Bida, Latitude 9^{0} 05', longitude 6^{0} 01') and host (sorghum, cultivar unknown) in the same year (1997), hereafter referred to as *S. hermonthica* BD 97. Besides, the seeds were subjected to uniform conditioning. The *S. hermonthica* seeds were characterised by a high germinability response to the synthetic strigol analogue, GR-24. All glassware and forceps were sterilised prior to pre-germination tests. The parasite seed were surface-disinfected for 5 min. in a 1% NaOCl. Floating seeds were discarded. The seeds were then thoroughly rinsed in three changes of sterile de-ionised water inside a funnel lined with Whatmann no. 2 filter paper (Whatman® Whatman Int. Ltd, maidstone, England), air-dried over a clean surface, and stored in glass vials at 28^{O}C. To condition, the dried *S. hermonthica* seeds were sprinkled on 3 mm diameter glass-fiber (GF/C, Whatman® Whatman Int. Ltd, maidstone, England) discs (30 - 40 seed/disc) that were placed on moistened filter paper in Petri-dishes. These were placed in an incubator (Gallenkamp, UK) in the dark at 28^{O}C for 14 d.

After incubation, the glass-fiber discs with the conditioned *S. hermonthica* seeds were removed from the Petri-dishes and placed in clean 9 cm diameter Petri-dishes. The discs were placed around a 2 cm diameter sterilised film canister filled with tempered Potato dextrose agar (PDA) (Difco) medium, centred in the Petri-dish, on freshly moistened filter paper. The discs were arranged in four radii forming a "cross" radiating from the film canister. Each line had four discs, with the first disk in each line touching the central canister.

Pure inoculums of the bacteria to be tested were aseptically streaked on the PDA. In negative controls, 300 µl of sterile deionised water was pipetted over uninoculated PDA as a substitute for bacterial inoculum. In the positive control, one parts per million (1 ppm) GR-24 was used. These were then incubated at 28^{O} C for 3 d and the percentage germination of *S. hermonthica* seeds on each disk was counted. Average percentage germination for each line of disks in each dish was calculated.

Screen-house experiments

Plantings were done in screen-house potted soil infested with *S. hermonthica*. Soil sterilisation was done at 100^{0}C for 4 h in Lindig (boiler) machine (Model ''SF'' Burner, MP1192, R.W. Beckett Corp. Elyria, Ohio, U.S.A). For the infested soil, *S. hermonthica* was added at a concentration of 500 seeds per 4 kg potted soil. The experimental site was at the International Institute of Tropical Agriculture (IITA) Ibadan in Oyo State, Latitude 7^{0} 43'N longitude 3^{0} 9'E Nigeria. Seeds of susceptible sorghum (varieties CK60B and Mokwa local) obtained from collections maintained at IITA were treated with bacterial isolates by pelleting with a mixture of bacterial cells from 24 h. old culture (5×10^{7} cfu ml^{-1} that was suspended in 1% methylcellulose (mc)). MC is effective as a bacterial preservative and does not significantly influence seed germination (Suslow and Schroth, 1981). Finely ground vermiculite, capable of passing through 300 mm mesh sieve was wrapped in aluminum foil and sterilised in the autoclave at 121^{O}C for 15 min. The vermiculite added to freshly inoculated wet seed in a beaker and mixed quickly for 2 min. until seed were evenly coated. The coated seed appeared as off-white and were allowed to dry overnight by spreading on a clean surface prior to sowing in sterilised potted soil infested with *S. hermonthica* BD 97. The sorghum seeds were sown immediately; and where this could not be done, the seeds were stored for not more than 3 weeks at low temperatures (4^{O}C) in a refrigerator. Seed, which were pelleted with a mixture, which did not contain bacterial cells (mc + powdered vermiculite), served as control.

Statistical analysis

Means of percentage germination were calculated for each Petri-dish (average of four lines) of each bacterium in each test. The data were analyzed using the UNIVARIATE procedure of SAS (Statistical Analysis System, SAS Institute, Cary, NC) to generate raw means and standard error. Test of the null hypothesis that the germination stimulated by each isolate equaled zero was done by employing the UNIVARIATE procedure. Analysis of variance for each isolate was done by the General Linear Model procedure of SAS. Experiments were repeated four times with four replicates each.

RESULTS

Isolation of rhizosphere bacteria

Forty isolates of bacteria were obtained from the rhizosphere of maize variety 8338-1 and 140 from rhizosphere of two cultivars of sorghum (CK60B and Mokwa local) grown in the screen- house at IITA Ibadan. Isolations were made from the exorhizosphere and endorhizosphere at biweekly intervals by destructive sampling. The bacterial isolates were evaluated for capability to stimulate germination of *S. hermonthica* BD 97. In a series of laboratory bioassays, a fluctuating (irregular) germination trend was observed in the *S. hermonthica* seeds as caused by the bacterial isolates. Some of the isolates were tentatively identified as fluorescent pseudomonads based on their water-soluble,

Table 1. Germination of *S. hermonthica* seeds from a population of sorghum plants from Bida, Nigeria, stimulated by selected rhizosphere bacteria.

Bacteria	% germination of *S. hermonthica* seeds [x]				mean[y]	Pr>t
	Dish 1	Dish 2	Dish 3	Dish 4		
Pseudomonas 10MKR4	1.751	0.001	0.001	0.001	0.4378	0.7591[z]
K. oxytoca 10MKR7	2.351	3.701	0.001	3.381	2.3586	0.1066
Pseudomonas 4MKS8	10.611	0.001	0.001	1.591	3.0510	0.0398
Ent. sakazakii 8MR5	1.211	0.001	2.061	0.001	0.8185	0.5675
GR-24	72.801	57.201	68.101	61.891	64.9985	0.0001
Water	0.001	0.001	0.001	0.001	0.0010	1.0000
Std Error	1.41					
LSD 5%	2.59					
Prob. of F	0.0001					
Coef. of variation 37.25						

N = 16 for each day
GR-24 = +ve control (Strigol-analogues, a synthetic germination stimulant)
Water = -ve control
[x]Means based on four replications; each conducted on a separate dish; values presented are arcsine transformed.
[y]Germination least squares means for mean percent germination
[z]Probability of a greater T value, under the null hypothesis that the mean equaled zero, based on percentage germination data.

greenish-yellow fluorescent pigment when examined under the ultraviolet light. The standard identification system of 'Appareils et Procedes d'identification' (API) technique (API Systems, Biomerieux, SA, France) was used for biochemical tests.

In *in vitro* experiment, isolates, *Enterobacter sakazakii* 8MR5, *Pseudomonas* sp. 4MKS8 and 10MKR4, *Klebsiella oxytoca* 10MKR7 were found to be promising rhizosphere bacteria that could stimulate the germination of *S. hermonthica* (Table 1). *Pseudomonas* sp. 4MKS8 stimulated the germination of seed of *S. hermonthica* by 3%. The percentage germination of *S. hermonthica* seed induced by all the isolates (Table 1) was weak. Consequently, they were tagged low-level germination isolates.

K. oxytoca 10MKR7 stimulated the germination of *S. hermonthica* seed to a limited degree, while the strigol analogue GR-24 induced effective germination in all replications with an average of 65% germination at a concentration of 1 ppm. Germination of *S. hermonthica* in water (control) was 0% in all tests. It was observed that germination percentages obtained with GR-24 solution vary from test to test despite the standard procedure used.

Screen-house experiments

The incidence of *Striga* plants over 9 week duration was recorded (Table 2) . *Striga* emergence was significantly stimulated over the control (water check). Throughout the entire experimental period, *S. hermonthica* emergence on the control pots was used as the standard for comparison. Percentage *Striga* emergence over the control pots showed that seed treatment with *Pseudomonas* sp. 10MKR4 gave the highest number of emerged *Striga*. This happened to be the least stimulant producer in the laboratory experiment followed by *Pseudomonas* sp. 4MKS8. Laboratory findings of *Pseudomonas* sp. 4MKS8 and *E.sakazakii* 8MR5 agreed with their screen-house findings.

DISCUSSION

This study demonstrates that there are some potential in certain rhizosphere bacteria to induce germination of *S. hermonthica*. This is in accordance with the observations of Babalola and Berner (2004) on length of the after-ripening period for *S. hermonthica*. The fact that the seeds were germinable, indicated that the synthetic strigol, GR- 24, induced effective germination of *S. hermonthica* seeds. The low stimulation of *S. hermonthica* by the bacterial isolates may be explained by the diffusion rates of the stimulant produced by the isolates. However, from the data, it was clear that GR - 24 is more effective than all the bacterial isolates in *S. hermonthica* seed germination stimulatory activity. That notwithstanding, the need for indigenous microbes in the integrated control method can not be overemphasized. The isolates tested could be low stimulant producers, such that, even statistically only stimulant from *Pseudomonas* sp. 4MKS8 is significant in the laboratory trial, but it is also possible that the seeds of the strain of *S. hermonthica* BD 97 do not respond to the isolates. Germination stimulant which are specific for *S. hermonthica* strains have been reported by King and Zummo (1977) and Bebawi (1981). The isolates so far obtained were identified as low stimulant producers (Babalola et

Table 2. Stimulation of *S. hermonthica* as influenced by different rhizosphere bacteria applied by pelleted treatment on susceptible host plant sorghum (var. Mokwa Local).

Selected bacterial isolate used in seed treatment	Average number of emerged *Striga* per pot[x]	Highest *Striga* count per pot	Highest *Striga* count as % of count in water check
Uninoculated control	8.2	14.1	100.00
Pseudomonas 10MKR4	15.0	30.3	214.89
K. oxytoca 10MKR7	13.9	25.5	180.85
Pseudomonas 4MKS8	13.4	28.0	198.58
E. sakazakii 8MR5	12.6	27.4	194.33
LSD (0.05)	3.68		
CV (%)	36.3		

[x]The soil was infested with 0.05 g *Striga* (about 3,000 germinable seeds out of about 10,000 total number of seeds) seed pot^{-1} before sowing of sorghum seeds.

et al., 2006). When stimulant activity was tested, in both laboratory and pot experiments, the percentage germination effected by the stimulatory action of the bacteria was much lower than could be recommended for *S. hermonthica* control without any other control method. The purpose of this development was to determine the bacterial stimulants for *S. hermonthica*, to release germination stimulants into the rhizosphere, and thereby further inducing the germination of *S. hermonthica* and consequently depleting the *Striga* seed bank when applied during trap cropping. These organisms that stimulate *S. hermonthica* seed germination may probably explain *Striga* decline in those sites for which there is no plausible explanation.

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