2015 STUDIES OF PLANT GROWTH PROMOTING RHIZOBACTERIA FOR BIOLOGICAL CONTROL OF *MELOIDOGYNE INCOGNITA* ON COTTON

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Abstract

Meloidogyne incognita (Root-knot Nematode, RKN) is the most damaging pathogen on cotton and caused a total of 494,000 bales yield losses in cotton in the U.S. in 2014. The objective of this research is to evaluate the effect of six *Bacillus* Plant Growth Promoting Rhizobacteria (PGPR) strains and two combinations for biocontrol of RKN in cotton in the microplot and field conditions in Alabama. Microplot trials were conducted in Plant Science Research Center at Auburn University and field trials were conducted in two naturally infested fields, in central Alabama. Bacterial cell suspensions were applied by dripping method for microplot trials and in furrow spray for field trials at a concentration of 1×10^7 CFU/seed. Seeds treated with Abamectin, or Poncho/Votivo and water were used as controls. All the experiments were randomized complete block design with six or five replications for microplot or field trials respectively. Microplot results indicated that strains AP52 significantly reduced the RKN populations similarly to Abamectin at 48 days after planting (DAP) ($P\leq0.1$) and supported the third highest yield in seed cotton at harvest. In the field, strain AP209 significantly increased plant height at 40 DAP in both fields ($P\leq0.1$) and significantly reduced RKN population in PBU at 40 DAP ($P\leq0.1$). In both locations, seed cotton yield enhancement was similar between the bacterial strains and the chemical control Abamectin at harvest ($P\leq0.1$). Strains AP52 and AP209 are very promising candidates for RKN management and cotton yield enhancement in the field.

Introduction

Meloidogyne incognita (Root-knot Nematode, RKN) is the most damaging pathogen on cotton and caused a total of 494,000 bales yield losses in cotton in the U.S. in 2014 (Lawrence, et al. 2014). The increasing of the environmental awareness, the consumer's health consciousness, and the withdrawal of aldicarb (Temik 15 G), has driven an increase in examinations of possible new control agents including biologicals in nematode management (Xiang, 2014). Many studies have reported antagonistic activity of the PGPR strains especially *Bacillus* species against plant parasitic nematodes. Eleven *Bacillus* species including *B. thuringiensis* (Devidas and Rehberger 1992; Mohammed, et al. 2008), *B. firmus* (Castillo, et al. 2013), *B. megaterium* (Kloepper, et al. 1992), *B. pumilus* (Raupach and Kloepper, 1998), *B. penetrans* (Brown and Smart, 1985), *B. cereus* (Raupach and Kloepper, 1998), *B. subtilis* (Raupach and Kloepper, 1998; Siddiqui, 2006), *B. coagulans* (Tibugaril, et al. 2012), *B. polyinyxa* (Khan and Akram, 2000), *B. sphaericus* (Krechel, et al. 2002), *B. circulans* (Serfoji, et al. 2010) have been documented for RKN control. Among the species of *Bacillus*, *B. firmus* GB-126 was originally isolated in Israel and currently formulated as a seed treatment under the name VOTiVO or as a wettable powder under the name Nortica 5%WP for nematode control (Castillo, et al. 2013). The objective of this research was to evaluate six *Bacillus* PGPR strains including *B. subtilis* subsp. *subtilis*, *B. safensis*, *B. amyloliquefaciens*, and *B. mojavensis* and two combinations for their biological control potential of root-knot nematode and yield enhancement in cotton in the microplot and field conditions.

Materials and Methods

Bacterial cultures preparation

Vegetative cells of *Bacillus* PGPR grown on tryptic soy agar (TSA) plates at pH 7 and at 35˚C in the incubator for 24 hours were suspended with distilled sterile water. The concentrations of bacterial suspensions were adjusted to 1×10^7 CFU/ml.

Nematodes preparation

Meloidogyne incognita nematode inoculum of for all tests were maintained by increasing the populations on corn in the greenhouse. Eggs of *M. incognita* were extracted from the corn roots by shaking the root system in a 1% NaOCl solution for 4 mins at 120 rpm (Castillo, et al. 2013). Egg suspension were collected on a 25-µm-pore sieve and

centrifuged by sucrose centrifugation-flotation method (Hussey and Barker, 1973). Eggs were enumerated at ×40 magnification with an inverted TS100 Nikon microscope and standardized to 10,000 per pot for microplot test.

Microplot experiment

Microplot trials were conducted in the Plant Science Research Center of Auburn University using 7 gallon pots in 2015. Pots were filled with a kalmia loamy sand (80% sand, 10% silt, and 10% clay) soil from the Plant Breeding Unit at E.V. Smith Research Center. Each pot was planted with four cotton seeds with the susceptible variety of "FM1944 GLB2" and was inoculated with 10,000 RKN eggs when planting. A 1 ml bacterial suspension with the concentration of 1×10^7 CFU/ml was applied to each seed at planting. Seeds treated with Abamectin Poncho/Votivo, and water were used as controls. Plant height, shoot fresh weight (SFW), root fresh weight (RFW), and eggs per gram of root were measured and counted at 48 day after planting (DAP). Seed cotton yield was obtained at harvest.

Field experiment

The field trials were established in two naturally RKN infested fields including Plant Breeding Unit (PBU) with soil type of kalmia loamy sand (80% sand, 10% silt, and 10% clay) and Prattville Agricultural Research Center (PARC) with sandy clay loam (64% sand, 10% silt, and 26% clay) at Auburn University in 2015. Plots were consisted of 2 rows, 7 m long with 0.9 m row spacing. Each row was planted with 100 cotton seed with the variety of FM1944 GLB2. Bacterial cell suspensions were applied as in furrow spray for field trials when planting with the concentration of 1×10^7 CFU/ml on each seed. Seeds treated with Abamectin Poncho/Votivo, and water were used as controls. Plant height, biomass including SFW and RFW, and eggs per gram of root were measured and counted at 40 DAP. Seed cotton yield was harvested at plant maturity.

Experimental Design

All the experiments were arranged in a randomized complete block design with six replications for microplot trials and five replications for field trials. Data collected were analyzed in SAS 9.4 (SAS Institute, Inc.) using Glimmix procedure and means were separated by Tukey's method with $P \leq 0.10$.

Results and Discussion

Microplot results

Microplot results indicated that the plant height (PH), shoot fresh weight (SFW), and root fresh weight (RFW) were similar at 48 DAP among all the *Bacillus* strains tested (Table 1). Strain AP52 supported similar RKN populations as Abamectin and were significantly lower than the water control RKN population at 48 DAP (Table 1). Seed cotton yield was similar among all the treatments at harvest but did vary by 59.3 g. Strains AP136, the combination of Abamectin+AP52+AP283 and strain AP52 ranked 1st, 2nd, and 3rd in seed cotton yield in the microplot trial producing 34%, 33% and 25% increased seed cotton yield compared with the water control (Table 1).

Table 1. Microplot plant height, shoot fresh weight, root fresh weight, eggs per gram of root (eggs/g of root) at 48 DAP, and seed cotton yield at harvest.

Treatment	Scientific name	48 DAP				136 DAP
		PH /cm	SFW/g	RFW/g	Eggs/g of root	Seed cotton yield /g
AP52	*Bacillus subtilis* subsp. *subtilis*	49.0	76.2	9.3	162.8 b	214.6
AP278	*Bacillus subtilis* subsp. *subtilis*	39.0	51.5	7.0	409.3 ab	178.2
AP283	*Bacillus safensis*	47.0	85.9	9.2	871.7 ab	185.3
AP136	*Bacillus amyloliquefaciens*	43.0	61.2	6.2	1357.2 ab	231.2
AP209	*Bacillus mojavensis*	51.0	89.0	10.1	211.7 ab	198.9
AP279	*Bacillus subtilis* subsp. *subtilis*	43.0	58.7	6.6	298.8 ab	189.8
AP52+283		44.0	57.7	5.2	912.7 ab	196.6
Abamectin+52+283		46.0	59.1	6.7	360.7 ab	228.8
Water		42.0	85.7	7.6	1551.3 a	171.9
Poncho/Votivo		48.0	59.5	7.4	435.7 ab	207.9
Abamectin		42.0	72.8	8.5	69.2 b	172.7

Means followed by the same letter do not significantly differ according to Tukey's method ($P \leq 0.10$).

Field results

Field test trials indicated that strain AP209 significantly increased plant height, biomass including shoot fresh weight and root fresh weight at 40 DAP in both field locations including the PBU and PARU of Auburn University (Table 2). Strain AP209 increased plant height and biomass of the cotton in both locations near 40 DAP. Also, Strain AP209 supported fewer RKN eggs and J2's in PBU, while strains AP52, AP278, and the combination Abamectin+52+283 significantly reduced RKN population at the PARU at 40 DAP (Table 2). In PBU, seed cotton yield was similar among all the treatments but varied by 836.9 lbs/a. Abamectin supported the highest yield in seed cotton followed by strain AP52 and AP209. At PARU, Abamectin significantly increased seed cotton yield over AP 278 although all AP strains were similar in yield to the water control (Table 2).

Table 2. Plant height, biomass including SFW and RFW, eggs per gram of root (eggs/g of root) at 40 DAP, and seed cotton yield at harvest in both PBU and PRAU of Auburn University.

Treatment	40 DAP						142 DAP	
	Plant height/cm		Biomass/g		Eggs/g of roots		Yield lbs/A	
	PBU	PARU	PBU	PARU	PBU	PARU	PBU	PARU
AP52	30.8 ab	15.3 ab	107.9 abc	23.2 ab	2229.2 a	602.4 b	3446.5	3884.1 ab
AP278	29.4 ab	15.8 ab	96.9 abc	23.6 ab	1943.6 ab	630.0 b	3154.9	3463.0 b
AP283	26.1 ab	13.6 b	96.9 abc	20.8 b	1980.2 a	1514.4 ab	3007.4	4065.6 ab
AP136	25.9 b	15.4 ab	74.8 c	25.2 ab	2118.0 a	3491.6 ab	2892.4	4276.1 ab
AP209	33.0 a	17.8 a	131.3 a	32.4 a	210.4 b	2429.6 ab	3268.7	4094.6 ab
AP279	30.6 ab	16.7 ab	105.5 abc	28.4 ab	1327.8 ab	8030.2 a	3181.6	4029.3 ab
AP52+283	26.3 ab	14.6 ab	81.7 bc	20.4 b	2737.4 a	1645.2 ab	3120.1	3927.7 ab
Abamectin+52+283	29.3 ab	16.7 ab	129.0 ab	26.8 ab	1108.2 ab	599.8 b	2815.7	4247.1 ab
Water	29.6 ab	15.7 ab	106.2 abc	24.4 ab	2519.0 a	3125.8 ab	2656.3	3621.2 ab
Poncho/Votivo	32.5 ab	15.0 ab	121.0 abc	22.8 ab	2594.2 a	1869.4 ab	2941.2	4334.2 ab
Abamectin	31.9 ab	15.1 ab	109.4 abc	26.2 ab	1016.4 ab	3260.0 ab	3492.9	4443.1 a

Means followed by the same letter do not significantly differ according to Tukey's method ($P \leq 0.10$).

Summary

In this study, the strain AP52 (*B. subtilis* subsp. *subtilis*) significantly reduced RKN population in the microplot and in PARU ($P \leq 0.10$). Strain AP209 (*B. mojavensis*) increased plant height, biomass in both field locations at an early stage. Strain AP209 (*B. mojavensis*) reduced RKN population in the microplot which was similar as strain AP52 and Abamectin also significantly reduced RKN population in PBU ($P \leq 0.10$). Both AP52 and AP209 showed similar effects as Abamectin on seed cotton yield enhancement. Strains AP52 and AP209 are very promising candidates for RKN management. More field studies need to be done to confirm the results.

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