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HARPINEA GENE TRANSGENIC COTTON REDUCES REPRODUCTION BY ROOT KNOT NEMATODES

Ned M. French
Eden Bioscience Corporation
Little Rock, AR
T.L. Kirkpatrick
University of Arkansas
Hope, AR
Zhongmin Wei
Eden Bioscience, Corp.
Bothell, WA

Abstract

A study was initiated to examine the effects transgenically expressed harpin$_{Ea}$ constructs on cotton infected with root-knot nematodes (RKN), *Meloidogyne incognita*. All plants were grown in a growth room environment located at the Southwest Education and Research Center near Hope, AR. At planting, each pot was inoculated with 3,000 RKN eggs. The first set of transgenically expressed harpin$_{Ea}$ constructs (PE58) were evaluated in a growth room experiment conducted in 2003 and 2004. In the subsequent experiment, six new constructs were tested, and three promising constructs were pulled forward from the initial experiment. All plants were grown in a growth room environment. Trials were terminated at approximately 45 days after initiation. Plant measurements and numbers of root knot nematode eggs and juveniles were recorded. In each experiment, select transgenic cultivars averaged 56% to 62% fewer eggs and 72% to 81% fewer juveniles compared with the Coker control cultivar. These results suggest that harpin$_{Ea}$ transgenic cotton may be a useful tool in reducing numbers of nematodes infesting cotton.

Introduction

Harpin$_{Ea}$ is a naturally occurring harpin protein from a novel group of compounds that was first reported from *Erwinia amylovora* (Wei et al., 1992). Harpin proteins, including harpin$_{Ea}$, elicit the expression of genes involved in the hypersensitive response and plant growth enhancement and activate an induced systemic defense response that has been associated with enhanced resistance in plants to pathogens and certain other pests (Wei & Beer, 1996). An outcome of these findings is the development of commercial Plant Health Regulator products containing harpins, such as **Messenger® STS**, **N-HIBIT™ CST**, and **ProAct™**.

Technologies originating from harpin proteins have been branded **Harp-N-Tek™**. Harpin$_{Ea}$ and other harpin proteins activate the natural stress-defense and growth responses in plants that increase plant growth, stamina, and vigor, improve overall plant health, and can lead to improved output quality, increased marketable yields, and enhanced shelf-life. Other desirable attributes of Harp-N-Tek products include low use rates, rapid degradation in the environment, little or no dietary exposure, negligible toxicity, ease of application, and mass production using simple, environmentally friendly, and cost-effective water-based fermentation technology. Harp-N-Tek products are formulated with low risk inert ingredients. The next step with harpin$_{Ea}$ was to introduce the gene into cotton to create harpin$_{Ea}$ gene transgenic cotton.

Plant-parasitic nematodes are a major production hindrance for cotton (*Gossypium hirsutum* L.) in many parts of the world. Root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood can be a major production constraint (Thomas & Kirkpatrick, 2002) **(Figure 1)**. Foliar application of harpin$_{Ea}$ to field-grown cotton where a significant *M. incognita* population was present resulted in improved seed cotton yield in Arkansas (French, 2001). The primary objective of this study was to determine if transgenic use of the harpin$_{Ea}$ gene influences the reproduction of *M. incognita* on cotton. Here we report two experiments evaluating transgenic expression of harpin$_{Ea}$ against RKN.

Materials & Methods

In two growth chamber experiments at the University of Arkansas Southwest Research Extension Center near Hope, AR, transgenically expressed harpin$_{Ea}$ protein was evaluated for effects on reproduction of *M. incognita* and growth of cotton. Cottonseed that transgenically expressed harpin$_{Ea}$ constructs were obtained from Eden Bioscience Corporation (Bothell, WA). The parent cotton variety used in the transformation was Coker 312. In experiment 1, which was conducted 11-December-2003 to 9-February-2004, eight transformed cotton lines that expressed harpin protein in construct PE58 were tested. Experiment 2 was conducted in the fall of 2005. Six new constructs (C14-1B3, C14-3B4, C15-3B4, C15-3B2, C18-1C3, C21-2B) and three PE58 constructs from experiment 1 (c58-9-14-1, c58-9-14-6, and p58-16-4) were tested. In both experiments, a diurnal regime of 13 hr. daylight (28-29°C) and 11 hr darkness (25-26°C) was used. A loamy sand field soil amended with fine silica sand (85% sand, 13% silt, and 2% clay, < 2% OM; pH 6.1) was fumigated with methyl bromide and then used in each test. Nematodes (*M. incognita* host race 3) were obtained from tomato stock cultures by NaOCl extraction (Hussey & Barker, 1973). Cottonseed was planted singly into 10.2 cm-d clay pots, and fungicide and nematicide treatments were not applied at planting. At planting, pots were infested by pipetting 5×10^3 eggs into three holes (0.5 cm-d × 2.5 cm deep) around the seedling. Plants were watered daily and fertilized as needed for vigorous growth. Experimental design was completely randomized and all treatments were replicated 10 times in each experiment.

At the completion of each test, plant weights were recorded, and root systems were removed carefully from the pots and separated from the soil. Numbers of second stage juveniles were assayed from the soil in each pot by semi-automatic elutriation (Byrd et al., 1976) and centrifugal flotation (Jenkins, 1964). Twenty egg masses were selected arbitrarily, collected in small vials, and processed with NaOCl to determine the number of eggs per female. Each root system was then processed with NaOCl to determine total eggs per plant. Analysis of variance was conducted on all data, and means were compared by Duncan's Multiple Range Test at P=0.05.

Results & Discussion

Experiment 1. Four constructs that transgenically express harpin$_{Ea}$ protein averaged 41% to 81% fewer *M. incognita* (root knot nematode, RKN) eggs compared with control plants (Coker variety) **(Table 1),** and reproduction of *M. incognita* was highest on control plants. Although all constructs averaged fewer eggs per root weight compared with Coker, these differences were not significant (P=0.05). Constructs c58-9-14-1, p58-16-4, and c58-9-14-6 averaged 43% to 56% less eggs and 41% to 78% fewer juveniles than the control, and these promising constructs were carried forward for continued testing.

Experiment 2. Construct c15-3B4 averaged 62% fewer *M. incognita* eggs than the control (Table 2), and all other constructs averaged less than a 30% reduction in numbers of eggs. Three constructs c15-3B4, c58-9-14-6, andc15-3B2 averaged 37% to 72% fewer juveniles than the control. The strongest construct appeared to be c15-3B4, which had 62% and 72% less eggs and juveniles, respectively, compared with the Coker control.

Conclusions

In two well-controlled growth chamber experiments, some of the constructs that transgenically express harpin$_{Ea}$ adversely influenced *M. incognita* population development. Reductions in egg and juvenile densities of up to 62% and 81% were observed. Repeated tests of three promising constructs provided mixed results. However, one construct that was initially tested in experiment 2 offered promising results against eggs and juveniles.

A reduced fecundity of female root-knot nematodes in plants that contain the harpin$_{Ea}$ gene as well as a subsequent lowering of juvenile establishment is interesting and may indicate that this protein could result in a change in the suitability of cotton as a host for the nematode. Previously reported findings with externally applied harpins evaluated in greenhouse trials (Kirkpatrick et. al 2003) demonstrated reductions in numbers of *M. incognita* and *Rotylenchulus reniformis* nematode eggs. Further study into transgenic harpin$_{Ea}$ cotton is merited to identify a promising and reliable cultivar.

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Literature Cited

Bednarz, C.W., Brown, S.N., Flanders, J.T., Tankersley, T.B., and Brown, S.M. 2002. Communications in Soil Science and Plant Analysis 33:933-945.

Byrd, D.W., Jr., Barker, K.R., Ferris, H., Griffin, W.E., Small, R.H., Stone, C.A., and Nusbaum, C.J. 1976. Two semi-automatic elutriators for extracting nematodes and certain fungi from soil. Journal of Nematology 8:206-212.

French, N. 2001. Messenger®- harpin protein use in cotton production. *Proceedings* 2001 Beltwide Cotton Conferences. National Cotton Council of America. Memphis, TN.

Hussey, R.S. and Barker, K.R. 1973. A comparison of methods of collecting inocula for *Meloidogyne* spp., including a new technique. Plant Disease Reporter 57:1025-1028.

Jenkins, W.R. 1964. A rapid centrifugal-flotation technique for extracting nematodes from soil. Plant Disease Reporter 48:692.

Kirkpatrick, T. L., J. R. Rich, & N. M. French II. 2003. Effect of Messenger and EBC-583 on Cotton Plant Growth and the Reproduction of *Meloidogyne incognita* and *Rotylenchulus reniformis*. APS 2003 Annual Meeting, Charlotte, NC. APS Abstracts of Presentations.

Thomas, S.H. and T.L. Kirkpatrick. 2001. Root-knot nematodes. Compendium of Cotton Diseases, Second Edition. T.L. Kirkpatrick and C.S. Rothrock, eds. American Phytopathological Society. St. Paul, MN.

Wei, Z. and Beer, V. 1996. Harpin from *Erwinia amylovora* induces plant resistance. Acta Horticulturae 411:221-224.

Table 1. ***Meloidogyne incognita*** **juveniles and eggs on Harpin$_{Ea}$ transformed varieties and Coker, untreated control variety. First experiment, 2004.**

Treatment	No. Juveniles / 500cc	% Reduction below Control[1/]	No. Eggs / g root weight	% Reduction below Control[1/]
CONTROL (Coker)	1,011		14,240	
c58-9-14-1[2/]	599	41%	6,258	56%
p58-19-7	192	81%		
p58-16-4[2/]	493	51%	7,170	50%
p58-19-3				
p58-5-10				
p58-20-1			9,887	31%
c58-9-14-6[2/]	226	78%	8,189	43%
p58-9b-14			6,291	56%

[1/] Constructs averaging at least 30% less eggs or juveniles are presented.
[2/] Denotes construct carried forward for second experiment.

Table 2. ***Meloidogyne incognita*** **juveniles and eggs on Harpin$_{Ea}$ transformed varieties and Coker, untreated control variety. Second experiment, 2005.**

Treatment	No. Juveniles / 500cc	% Reduction below Control[1/]	No. Eggs / g root weight	% Reduction below Control[1/]
CONTROL (Coker)	2,211		5,104	
c15-3B4	630	72%	1,931	62%
c58-9-14-1				
p58-16-4				
c58-9-14-6	1,397	37%		
c18-1C3				
c21-2B1				
c14-1B3				
c14-3B4				
c15-3B2	1,153	48%		

[1/] Constructs averaging at least 30% less eggs or juveniles are presented.

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