RESEARCH

Expression of *cry1Fa* in Bahiagrass Enhances Resistance to Fall Armyworm

Gabriela Luciani, Fredy Altpeter,* Jessica Yactayo-Chang, Hangning Zhang, Maria Gallo, Robert L. Meagher, and David Wofford

ABSTRACT

Bahiagrass (*Paspalum notatum* var. *saurae*) is the predominant forage grass in Florida and in other subtropical regions. To improve pest resistance against fall armyworm [*Spodoptera frugiperda* (J. E. Smith)], an optimized *cry1Fa* gene encoding a δ-endotoxin from *Bacillus thuringiensis* was synthesized, subcloned under the transcriptional control of the constitutive *ubi1* promoter, and introduced into the bahiagrass cultivar Tifton 9 by particle bombardment. Three transgenic bahiagrass lines were generated using minimal transgene expression cassettes without vector backbone. Southern blot analyses showed independent *cry1Fa* transgene integration patterns for the three lines. Transcripts of *cry1Fa* were detected in all three transgenic lines by reverse transcriptase polymerase chain reaction. Cry1Fa protein was detected in two lines by immuno-chromatography and quantitative Cry1Fa enzyme linked immunosorbent assay (ELISA). The Cry1Fa ELISA also indicated stable *cry1Fa* transgene expression in vegetative progeny plants of both lines. *Cry1Fa* expression levels correlated well to resistance levels determined by insect bioassays. An average mortality rate of 83% was observed when neonate larvae of fall armyworm were fed with transgenic leaves of the highest *cry1Fa* expressing line. These results indicate that minimal expression cassette technology supports stable and high level expression of *cry1Fa* in bahiagrass which can control fall armyworm, a devastating pest of forage grasses.

G. Luciani, F. Altpeter, J. Yactayo-Chang, H. Zhang, M. Gallo, and D. Wofford, Univ. of Florida-IFAS, Agronomy Dep., Genetics Institute, Plant Molecular and Cellular Biology Program, PO Box 110300, Gainesville, FL 32611; R.L. Meagher, USDA, ARS, CMAVE, Gainesville, FL 32608-1069. Received 6 Apr. 2007. *Corresponding author (faltpeter@ifas.ufl.edu).

Abbreviations: BAP, benzylaminopurine; Bt, *Bacillus thuringiensis;* cry, crystal protein; CaMV, Cauliflower Mosaic Virus; CIM, callus induction medium; Dicamba, 3,6-Dichloro-2-methoxy benzoic acid; ELISA, enzyme linked immunosorbent assay; IPM, integrated pest management; MC, minimal transgene expression construct; *nptII,* neomycin phosphotransferase II; MS, Murashige and Skoog; PCR, polymerase chain reaction; RT–PCR, reverse transcriptase polymerase chain reaction.

Bahiagrass (*Paspalum notatum* var. *saurae*) is an important forage grass in tropical and subtropical regions around the world. It is grown on 2.5 million ha in the southern United States (Burton et al., 1997; Blount et al., 2001). Its popularity is based on low maintenance requirements and tolerance to drought, heat, many diseases, and overgrazing (Chambliss, 2002). However, bahiagrass is susceptible to two major insect pests: mole crickets (*Scapteriscus* spp.) and fall armyworm [*Spodoptera frugiperda* (J. E. Smith)]. Fall armyworm is one of the most important insect pests in the southeastern United States, causing significant seasonal economic losses in forage and turf grasses and many other crops (Sparks 1979, Meagher and Nagoshi, 2004; http://www.bugwood.org; verified 10 Sept. 2007). Fall armyworm refers to two host strains that differ in host preference, physiology, behavior, and pesticide susceptibility (Nagoshi and Meagher, 2004). The corn strain is associated with maize

Published in Crop Sci. 47:2430–2436 (2007).
doi: 10.2135/cropsci2007.04.0195

677 S. Segoe Rd., Madison, WI 53711 USA

(*Zea mays* L.) and sorghum [*Sorghum bicolor* (L.) Moench], while the rice (*Oryza sativa* L.) strain is found preferentially in rice, turf grasses, and pasture grasses. The two host strains are morphologically identical, and can only be reliably distinguished by molecular methods (Nagoshi and Meagher, 2004, Nagoshi et al., 2006).

Traditionally, many insect pests are controlled using integrated pest management (IPM) strategies involving the use of pesticides with resistant varieties or biological control agents. However, the indiscriminate use of pesticides produces adverse effects on human health and the environment including the development of insect resistance and the elimination of other beneficial insects (Ranjekar et al., 2003; Ferry et al., 2006). Transgenic crops expressing *Bacillus thuringiensis* (*Bt*) δ-endotoxins were a natural choice for controlling insects since *Bt* crystal protein (Cry) and spore formulation products have been successfully used for many years (Schnepf et al., 1998; Ferre and Van Rie, 2002; Ranjekar et al., 2003; Kaur, 2006).

Insect resistance to *Bt* toxins in targeted populations arises through different mechanisms and/or at different levels (Ferre and Van Rie, 2002; Griffitts and Aroian, 2005). Therefore, to delay insect resistance development, growers must conform to the "High-dose refuge strategy". The first component of this strategy is to express toxins in plants at a high enough level to kill heterozygotes in the insect population. To increase *Bt* expression levels in transgenic plants, codon-optimization of *cry* sequences, the reduction of AT sequences and the truncation of the native *cry* sequence have been successfully used (Schnepf et al., 1998; Bohorova et al., 2001; Kaur, 2006). Elimination of vector backbone sequences and biolistic transfer of minimal transgene expression constructs (Fu et al., 2000) also supported high transgene expression levels (Agrawal et al., 2005). Stacking of different *cry* genes (Kaur, 2006), expression of *cry* fusion constructs (Bohorova et al., 2001), and pyramiding genes including *cry* genes with genes encoding proteins having alternative insect control mechanisms like vegetative insecticidal proteins or proteinase inhibitors, will reduce the risk of insects developing resistance to *Bt* toxins (Ferry et al., 2006). The second component of the "High-dose refuge strategy" is to provide structured refuges. Refuges are small areas cultivated with nontransgenic crops which are interspersed with the transgenic crop. Mating of susceptible adults that developed from nontransformed plants with those from transgenic plants allow for the elimination of homozygous resistant individuals and the reduction of resistant alleles (Cannon, 2000; Ranjekar et al., 2003).

Currently, *Bt* transgenic technology is adopted worldwide and *Bt* crops are grown on more than 14 million hectares (James, 2005). In the United States, *Bt* crops are grown on approximately 20% of the crop acreage and their use is directly linked to higher yields and profits and reduced pesticide application (Cannon, 2000). Currently, marketed products include *Bt* corn containing the *cry1Ab*, *cry1Fa*, *cry3Bb1,* and stacked *cry1Ab* and *cry3Bb1* genes for controlling European corn borer (*Ostrinia nubilalis* Hübner), southwestern corn borer (*Diatraea grandiosella* Dyar) and corn rootworm (*Diabrotica barberi* Smith and Lawrence), and *Bt* cotton (*Gossypium hirsutum* L.) containing *cry1Ac*, stacked *cry1Ac* and *cry2Ab2,* stacked *cry1Ac,* and *cry1Fa* for controlling tobacco budworm (*Heliothis virescens* Fabricius), cotton bollworm (*Helicoverpa zea* Boddie), and pink bollworm (*Pectinophora gossypiella* Saunders) (Castle et al., 2006). Cry1Fa has been reported to control fall armyworm in cotton (Adamczyk and Gore, 2004). However, there are no previous reports on Cry proteins expressed in forage and turf grasses and their effects against fall armyworm. Transgenic plants of the noncommercial apomictic genotype 'Tifton 7', diploid bahiagrass cultivar Pensacola, and apomictic cultivar Argentine have been recently reported (Smith et al., 2002; Gondo et al., 2005; Altpeter and James, 2005; Sandhu et al., 2007). These genetic transformation protocols allow the introduction of exogenous insect resistance genes into bahiagrass. Hence, the objective of this work was to evaluate the expression of a synthetic *cry1Fa* gene in transgenic bahiagrass and its effect on resistance to fall armyworm.

MATERIALS AND METHODS

Minimal Transgene Expression Constructs

Based on the *cry1Fa* gene sequence available in the NCBI database (M73254), a codon-optimized sequence for the δ-endotoxin was generated. The synthetic *cry1Fa* gene (1863 bp) was synthesized and subcloned into a pPCR-Script vector by Geneart (Regensburg, Germany). Restriction sites *Bam*HI and *Hind*III were introduced at the 5′ and 3′ ends of the *cry1Fa* coding sequence, respectively to facilitate subcloning into vector pHZUbi-ox1 (Agharkar et al., 2007). The resulting pHZCRY vector contains the maize ubiquitin 1 promoter and first intron (Christensen et al., 1992), the *cry1Fa* coding sequence and the nos. 3′ untranslated region (Fraley et al., 1983) (Fig. 1).

The *npt*II selectable marker cassette contains the neomycin phosphotransferase II *(nptII*) coding sequence (Bevan, 1984) under transcriptional control of the Cauliflower Mosaic Virus (CaMV) 35S promoter (Odell et al., 1985) and hsp70 intron (Rochester et al., 1986), and the CaMV 35S polyadenylation signal (Dixon et al., 1986) (Fig. 1). Following the strategy described by Fu et al. (2000), minimal transgene expression constructs (MCs) containing only the expression cassettes without vector backbone were used for biolistic gene transfer. The *npt*II and *cry1Fa* gene expression cassettes were excised from their plasmids by restriction digestion with *Not*I resulting in a 2.55- or 4.15-kb fragment, respectively (Fig. 1). Transgene expression cassettes were isolated by gel electrophoresis, and the corresponding band was excised and purified using the Wizard SV Gel and polymerase chain reaction (PCR) cleanup system (Promega, Madison, WI).

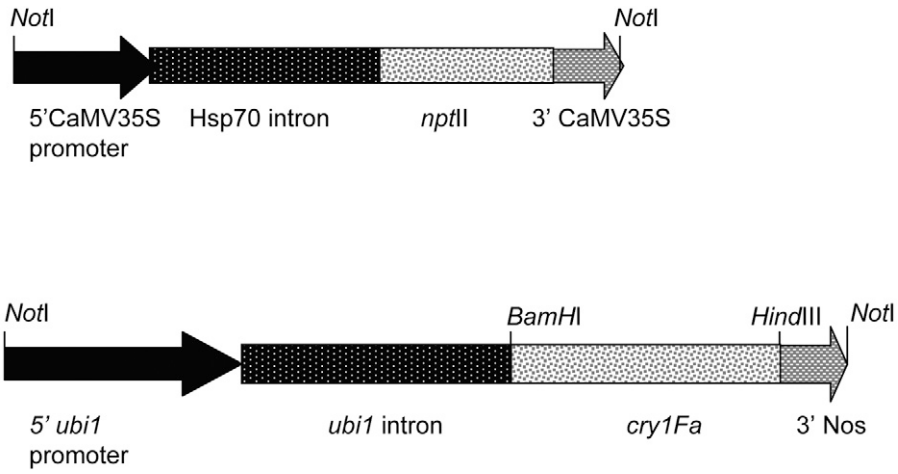

Figure 1. Expression cassettes used for the generation of transgenic bahiagrass by biolistic gene transfer. Schematic representation of the minimal expression cassettes for *npt*II (2554 bp) excised from vector pHZ35SNPTII (top) and the synthetic *cry1Fa* gene (4155 bp) excised from vector pHZCRY (bottom).

Tissue Culture, Transformation, and Regeneration of Bahiagrass

Embryogenic callus was induced from mature seeds of the diploid bahiagrass cultivar Tifton 9 following a protocol described earlier (Altpeter and Positano, 2005). The callus induction medium (CIM) consisted of 4.3 g L^{-1} Murashige and Skoog (MS) salts (Murashige and Skoog, 1962), 30 g L^{-1} sucrose, 1.1 mg L^{-1} 6-benzylaminopurine (BAP), 3 mg L^{-1} 3,6-dichloro-2-methoxy benzoic acid (dicamba) and 6 g L^{-1} agarose (Sigma, St. Louis, MO), supplemented with filter sterilized MS vitamins (Murashige and Skoog, 1962) which were added after the medium was autoclaved for 20 min. Calli were kept in darkness at a temperature of 28°C and subcultured to fresh CIM after 2 wk. Embryogenic calli were placed on CIM medium supplemented with 0.4M sorbitol, for 4 to 6 h before gene transfer and 7 wk after culture initiation. The *npt*II and *cry1Fa* gene expression cassettes were used in a 1:2 molar ratio and coprecipitated on 1.0 μm diameter gold particles (Altpeter and James 2005). The BioRad PDS-1000/He device (BioRad Laboratories Inc., Hercules, CA) was used for biolistic gene transfer at 1100 psi and 28 mm Hg. Bombarded calli were transferred to fresh CIM following gene transfer, and kept in the dark for 10 d before being transferred to low intensity light conditions (30 μmol m^{-2} s^{-1}), with 16 h/8 h light/dark photoperiod, at 28°C, on selection CIM containing 50 mg L^{-1} of paromomycin. After 4 wk, calli were subcultured on shoot regeneration medium, similar to CIM but containing 0.1 mg L^{-1} BAP and no dicamba, and transferred to high light (150 μmol m^{-2} s^{-1}) intensity with a 16 h/8 h light/dark photoperiod at 28°C. After 2 wk, calli were transferred to hormone-free CIM to induce root formation. After 4 to 6 wk, regenerated plantlets were transplanted into Fafard 2 mix (Fafard Inc., Apopka, FL) and acclimatized in growth chambers at 400 μmol m^{-2} s^{-1} light intensity with a 16 h/8 h light/dark photoperiod at 28°C/20°C day/night. Two weeks later plants were transferred to an air-conditioned greenhouse at 30°C/20°C day/night and natural photoperiod. Plants were fertilized biweekly with Miracle-Gro Lawn Food (Scotts Miracle-Gro, Marysville, OH) at the recommended rate.

Polymerase Chain Reaction, Reverse Transcriptase Polymerase Chain Reaction, and Southern Blot Analysis

Genomic DNA was extracted from the transgenic lines and wild-type as described by Dellaporta et al. (1983). The forward primer 5′ATGGTTTCAACAGGGCTGAG3′ and the reverse primer 5′CCTTCAC-CAAGGGAATCTGA3′ were designed for amplifying a 570-bp fragment internal to the coding sequence of the *cry1Fa* gene. Approximately 100 ng genomic DNA was used as template for PCR in a BioRad Icycler (Bio-Rad Laboratories Inc., Hercules, CA). The PCR was performed using the HotStart PCR kit (Qiagen, Valencia, CA). The cycling conditions were 95°C for 15 min initial denaturation, 35 cycles of 95°C for 1 min, 57°C for 1 min, 72°C for 1 min and 72°C for 15 min final extension. The PCR products were analyzed by electrophoresis on a 0.8% agarose gel.

Total RNA was extracted from emerging young leaves using the RNeasy Plant Mini Kit (Qiagen, Valencia, CA), followed by RNase free DNase I (Qiagen, Valencia, CA) treatment to eliminate genomic DNA contamination. Total RNA (500 ng) was used for cDNA synthesis via reverse transcription with the iScript cDNA Synthesis kit (Bio-Rad Laboratories Inc., Hercules, CA) in a reaction volume of 20 μL. Complementary DNA (2 μL) was used as a template to detect the transcripts of the *cry1Fa* gene by PCR with the same primer pair as described above for PCR from genomic DNA. The PCR products were analyzed by electrophoresis on a 0.8% agarose gel.

For Southern blot analysis, genomic DNA from wild-type and transgenic lines was isolated using the CTAB method as described by Doyle and Doyle (1987). Genomic DNA (15 μg) was digested with *Bam*HI and fractionated on a 1% agarose gel, transferred onto a nitrocellulose membrane (Hybond, Amersham BioSciences, Piscataway, NJ) and hybridized using the complete *cry1Fa* coding sequence (1.8 kb) as a probe, labeled with ^{32}P using the Prime-a-Gene kit (Promega). Hybridization and detection were performed according to the instructions of the manufacturer.

Immunological Assays

Quantitative expression of the *cry1Fa* endotoxin in leaf tissue from the transgenic lines was evaluated using the Quickstix kit for *cry1Fa* (EnviroLogix, Portland, ME) originally developed for Herculex I corn and following the recommendations of the manufacturer. Relative levels of expression of the *cry1Fa* endotoxin in leaf tissue were estimated by using the enzyme linked immunosorbent assay (ELISA) QualiPlate kit for *cry1Fa* (EnviroLogix) originally developed for Herculex I corn. Protein extracts from *cry1Fa* expressing corn grain were quantified and used as a positive control in a dilution series. Following 8 mo of vegetative propagation of the primary transformants, protein extracts were obtained from wild-type and the three transgenic lines including three different vegetative clones per line and three different replicates per clone. Protein concentration of the extracts was determined using the

Bradford assay (Bradford 1976) and absorbance was measured at 595 nm. Bovine serum albumine was used to prepare a standard curve (R^2 value of 96%). Ten micrograms of total protein were loaded per well. The immunoassay was performed according to the instructions of the manufacturer. Reaction kinetics was recorded at 450 nm using an ELISA microplate reader (Bio-Rad Laboratories Inc., Model 680). Optical densitometry values for each line were compared within the linear range of the reaction kinetics after addition of the ELISA substrate.

Insect Bioassays

Insecticidal activity of the transgenic lines was evaluated by following a modified version of the protocol described by Adamczyk and Gore (2004). Fall armyworm neonates (rice host strain) were obtained from egg masses hatched the same day, placed in Petri dishes and fed on four leaf pieces of 2 cm length of the third fully emerged leaf. A completely randomized experimental design was used. There were 10 replications per transgenic line represented by individual Petri dishes with leaves and larvae, and the experiment was repeated four times. For estimating fall armyworm resistance, the survival rate from transgenic lines was compared to the wild-type after 5 d of feeding.

Statistical Analysis

Optical densitometry data resulting from Cry1F ELISA of wild-type and transgenic lines were analyzed by Proc Anova and means were separated according to Tukey's test ($P < 0.05$) (Littell et al., 1996, SAS Institute, 2002).

Survival rates of neonate larvae of fall armyworm, expressed as a percentage, were analyzed by Proc Mixed and means were separated according to Fisher's protected LSD (Littell et al., 1996, SAS Institute, 2002). Standard errors are shown in figures as vertical bars.

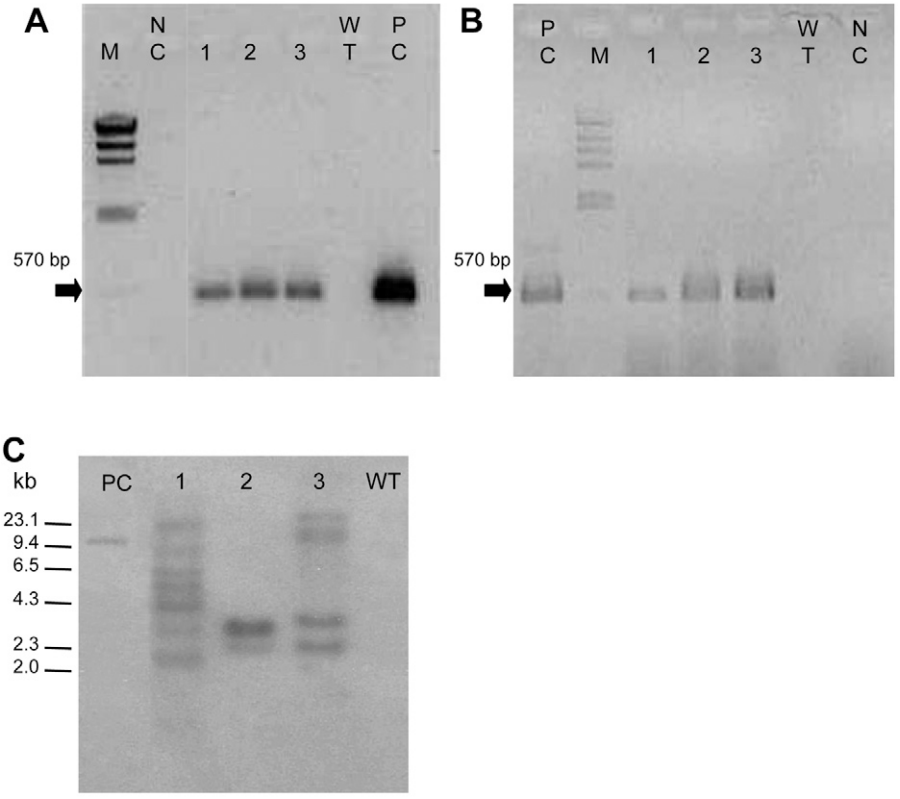

Figure 2. Polymerase chain reaction (PCR), reverse transcription polymerase chain reaction (RT–PCR), and Southern blot analysis of *cry1Fa* in bahiagrass. (A) A 570-bp fragment was amplified by PCR from genomic DNA of transgenic bahiagrass lines (1, 2, and 3) in comparison to wild-type (WT), buffer (NC), and pHZCRY plasmid DNA (PC) using the *cry1Fa* specific forward 5′ATGGTTTCAACAGGGCTGAG3′ and reverse 5′CCTTCACCAAGGGAATCTGA3′ primers. (M) lambda *Hind*III marker. (B) The presence of the *cry1Fa* transcripts following RT–PCR of bahiagrass lines (1, 2, and 3) in comparison to WT plasmid, NC, and PC using the *cry1Fa* specific primers as described above. (M) lambda *Hind*III marker. (C) Southern blot of genomic DNA from transgenic bahiagrass lines (1, 2, and 3), WT bahiagrass and 20 pg PC following hybridization with a radio-labeled probe of the complete *cry1Fa* coding sequence.

RESULTS

Generation of Transgenic Bahiagrass

Cobombardment of 300 'Tifton 9' calli with MCs of the pHZCRY and the pHZ35SNPTII vectors (Fig. 1) and selection on paromomycin-containing culture medium resulted in the regeneration of three independent transgenic lines. These lines were transferred to soil under controlled environment conditions and did not differ phenotypically from the wild type.

Molecular Characterization of Transgenic Bahiagrass

Initial screening for the presence of the *cry1Fa* gene by PCR analysis showed that all three regenerated bahiagrass lines contained the expected 570-bp *cry1Fa* fragment. This fragment also was amplified from the plasmid control and was absent in the wild-type bahiagrass (Fig. 2A). For expression analysis, reverse transcriptase polymerase chain reaction (RT–PCR), using the same *cry1Fa* specific primers, showed that the three lines amplified a 570-bp *cry1Fa* fragment, confirming the presence of *cry1Fa* transcripts in all transgenic plants (Fig. 2B).

Southern blot analysis showed an independent integration pattern for each transgenic line. Line 1 showed seven hybridization bands while lines 2 and 3 displayed two and four hybridization bands respectively (Fig. 2C).

A qualitative immuno-chromatographic assay showed that transgenic lines 2 and 3 contained Cry1Fa protein above the detection level of the kit. However, the Cry1Fa protein was not detected in protein extracts of line 1 (Fig. 3A). Therefore, a quantitative Cry1Fa ELISA was employed. This method revealed that line 1 produced relatively low levels of Cry1Fa. Lines 2 and 3 displayed *cry1Fa* expression levels that were 4- and 12-fold higher than those levels observed in line

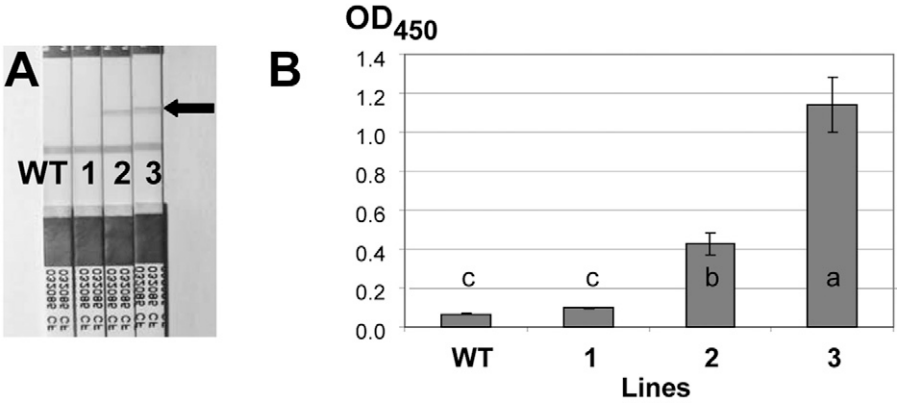

Figure 3. Cry1Fa protein expression in leaves of transgenic bahiagrass lines and wild type. (A) Immuno-chromothographic (cry1Fa Quickstix, Envirologix) evaluation of protein extracts of transgenic bahiagrass (1, 2, and 3) or wild type (WT). The arrow indicates the diagnostic band. (B) Optical densitometry at 450 nm (OD_{450}) values of Cry1Fa enzyme linked immunosorbent assay (ELISA) of protein extracts from transgenic bahiagrass lines (1,2, and 3) and wild-type bahiagrass (WT), on an average of triplicate analysis of three independent vegetative clones. Bars represent standard error. Letters indicate differences at $P < 0.05$ by Tukey test.

1, respectively ($P < 0.05$, Fig. 3B). Also, all three vegetatively propagated clones from these lines expressed the transgene. No significant differences in *cry1Fa* expression between clones of the same line were found ($P < 0.05$; data not shown). Cry1Fa protein levels in bahiagrass leaves were estimated by comparison with a crude Cry1Fa protein standard supplied by the manufacturer and were approximately 1.4 and 4.5 μg protein g^{-1} fresh weight for lines 2 and 3, respectively.

Insect Bioassays

Differences in feeding patterns were observed between larvae fed leaves from wild type and line 1 vs. lines 2 or 3 (Fig. 4A). Larvae fed consistently on leaves of line 1 or wild-type plants resulting in larger larvae and almost complete consumption of the leaves after 5 d. In contrast, most neonates foraged little on the leaves from lines 2 and 3, and died within the first three days of feeding (Fig. 4A).

Larvae fed leaves from lines 2 and 3 showed a significantly lower survival rate (35% and 17.5%, respectively), than wild type which had an 80% neonate survival rate. Larvae fed leaves of line 1, with low *cry1Fa* expression, did not differ significantly from those fed wild-type leaves ($P < 0.05$, Fig. 4B).

DISCUSSION

This is the first report of stable, transgene expression of a Bt crystal protein gene that confers insect resistance in a forage and turfgrass. Bahiagrass is an important subtropical forage grass that is also used as low input turf. Constitutive overexpression of the *cry1Fa* gene in bahiagrass resulted in increased resistance to fall armyworm.

Analysis of the complexity of MC integration patterns had resulted in controversial results in the past. Fu et al. (2000) described that biolistic transfer of MCs resulted in simpler integration patterns and lower copy numbers than plasmids. In contrast, no differences between the two DNA forms were reported by Breitler et al. (2002) and Romano et al. (2003). Southern blot analysis of the three transgenic bahiagrass lines transformed with MCs of the *cry1Fa* gene showed multiple transgene copies in all lines with line 1 displaying the most complex transgene integration pattern. This complex transgene integration pattern following biolistic transfer of MCs into bahiagrass is in agreement with findings of Breitler et al. (2002) and Romano et al. (2003). It suggests that the complexity of transgene integration is more likely dependent on factors intrinsic to the plant than on the form of DNA as proposed by Agrawal et al. (2005). Nevertheless, clean DNA technology by employing the use of MCs for biolistic transformation is capable of producing similar or higher transformation and expression efficiencies than whole plasmids (Agrawal et al., 2005). In the present study, two of the three lines expressed the *cry1Fa* transgene in vegetative progeny at a high enough level to control fall armyworm.

Transgenic corn expressing the Bt *cry1Ab* gene was first commercially released in 1996 (Castle et al., 2006). Constitutive *cry1Ab* expression of 3.3 or 10.3 μg g^{-1} fresh weight of leaves resulted in 50 to 75% or 98% control of the European corn borer (*O. nubilalis*) in corn field trials (Mendelsohn et al., 2003). This pest is considered the most important corn insect pest in the midwestern and northeastern regions of the United States (Wiatrak et al., 2004); while fall armyworm is the most important pest on grasses and other crops in the southeastern United States. Recently, corn expressing the *cry1Fa* gene (Herculex I) was commercially released by Pioneer Hi-Bred International and Dow Agrosciences (Events TC1507 and DAS-06275-8). Field trials indicated that these transgenic corn lines effectively controlled multiple insect pests like *O. nubilalis*, *D. grandiosella*, *H. zea*, *S. frugiperda*, *A. ipsilon,* and *R. albicosta* (USEPA, 2001). In cotton, fall armyworm bioassays indicated that neonate mortality was significantly higher when larvae were fed on leaves expressing *cry1Fa* (80%) compared with nontransgenic leaves (48%) or leaves expressing *cry1Ac* (45%) (Adamczyk and Gore, 2004). Cry1Fa concentrations were estimated at 1.4 and 4.5 μg g^{-1} fresh weight in bahiagrass transgenic lines 2 and 3, respectively. These expression levels were associated with 65 and 83% neonate mortality, respectively, while wild-type and transgenic bahiagrass with barely detectable *cry1Fa* expression showed a significantly lower fall armyworm mortality rate. These results indicate the potential

of *cry1Fa* to control fall armyworm in accordance with the results reported for *cry1Fa* expressing cotton (Adamczyk and Gore, 2004) and corn (USEPA, 2001).

In conclusion, stable expression of minimal synthetic *cry1Fa* expression constructs in bahiagrass conferred resistance to the difficult to control, and important insect pest, fall armyworm.

Acknowledgments

We would like to thank USDA/CSREES T-STAR and the University of Florida-IFAS for financial support. We also thank Jeff Seib for training in safe handling of radioisotopes.

References

Adamczyk, J.J., Jr., and J. Gore. 2004. Laboratory and field performance of cotton containing *cry1Ac, cry1F*, and both *cry1Ac* and *cry1F* (Widestrike®) against beet armyworm and fall armyworm larvae (Lepidoptera: Noctuidae). Fla. Entomol. 87:427–432.

Agharkar, M., P. Lomba, F. Altpeter, H. Zhang, K. Kenworthy, and T. Lange. 2007. Stable expression of *AtGA2ox1* in a low-input turfgrass (*Paspalum notatum* Flugge) reduces bioactive gibberellin levels and improves turf quality under field conditions. Plant Biotechnol. J. (in press).

Agrawal, P.K., A. Kohli, R.M. Twyman, and P. Christou. 2005. Transformation of plants with multiple cassettes generates simple transgene integration patterns and high expression levels. Mol. Breed. 16:247–260.

Altpeter, F., and V.A. James. 2005. Genetic transformation of turf-type bahiagrass (*Paspalum notatum* Flugge) by biolostic gene transfer. Int. Turfgrass Soc. Res J. 10:485–489.

Altpeter, F., and M. Positano. 2005. Efficient plant regeneration from mature seed derived embryogenic callus of turf-type bahiagrass (*Paspalum notatum* Flugge). Int. Turfgrass Soc. Res. J. 10:479–484.

Bevan, M. 1984. Binary agrobacterium vectors for plant transformation. Nucleic Acids Res. 12:8711–8721.

Blount, A.R., K.H. Quesenberry, P. Mislevy, R.N. Gates, and T.R. Sinclair. 2001. Bahiagrass and other *Paspalum* species: An overview of the plant breeding efforts in the Southern Coastal Plain. *In* Proc. 56th Southern Pasture and Forage Crop Improvement Conf., Springdale, AR.21–22 Apr. 2001. Available at http://www.spfcic.okstate.edu/proceedings/2001/breeders/blount.htm (verified 12 Sept. 2007).

Bohorova, N., R. Frutos, M. Royer, P. Estanol, M. Pacheco, Q. Rascon, S. McLean, and D. Hoisington. 2001. Novel synthetic *Bacillus thuringiensis cry1B* gene and the *cry1B-cry1Ab* translational fusion confer resistance to southwestern corn borer, sugarcane borer and fall armyworm in transgenic tropical maize. Theor. Appl. Genet. 103:817–826.

Bradford, M.M. 1976. Rapid and sensitive method for quantification of microgram quantities of protein utilizing principle of protein-dye binding. Anal. Biochem. 72:248–254.

Breitler, J.C., A. Labeyrie, D. Meynard, T. Legayre, and E. Guiderdoni. 2002. Efficient microprojectile bombardment-mediated transformation of rice using gene cassettes. Theor. Appl. Genet. 104:709–719.

Burton, G.W., R.N. Gates, and G.J. Gasho. 1997. Response of Pensacola bahiagrass to rates of nitrogen, phosphorus and potassium fertilizers. Soil Crop Sci. Soc. Fla. Proc. 56:31–35.

Cannon, R.J.C. 2000. Bt transgenic crops: Risks and benefits. Int. Pest Manage. Rev. 5:151–173.

Castle, L.A., G.S. Wu, and D. McElroy. 2006. Agricultural input traits: Past, present and future. Curr. Opin. Biotechnol. 17:105–112.

Chambliss, C.G. 2002. Bahiagrass. EDIS SSR-36. Agron. Dep., Coop. Ext. Serv., IFAS, Univ. of Florida, Gainesville.

Christensen, A.H., R.A. Sharrock, and P.H. Quail. 1992. Maize polyubiquitin genes: Structure, thermal perturbation of expression and transcript splicing, and promoter activity following transfer to protoplasts by electroporation. Plant Mol. Biol. 18:675–689.

Dellaporta, S.L., J. Wood, and J.B. Hicks. 1983. A plant DNA minipreparation: Version II. Plant Mol. Biol. Rep. 1:19–21.

Dixon, L., T. Nyffenegger, G. Delley, J. Martinez Izquierdo, and T. Hohn. 1986. Evidence for replicative recombination in Cauliflower Mosaic-Virus. Virology 150:463–468.

Doyle, J.J., and J.L. Doyle. 1987. A rapid DNA isolation

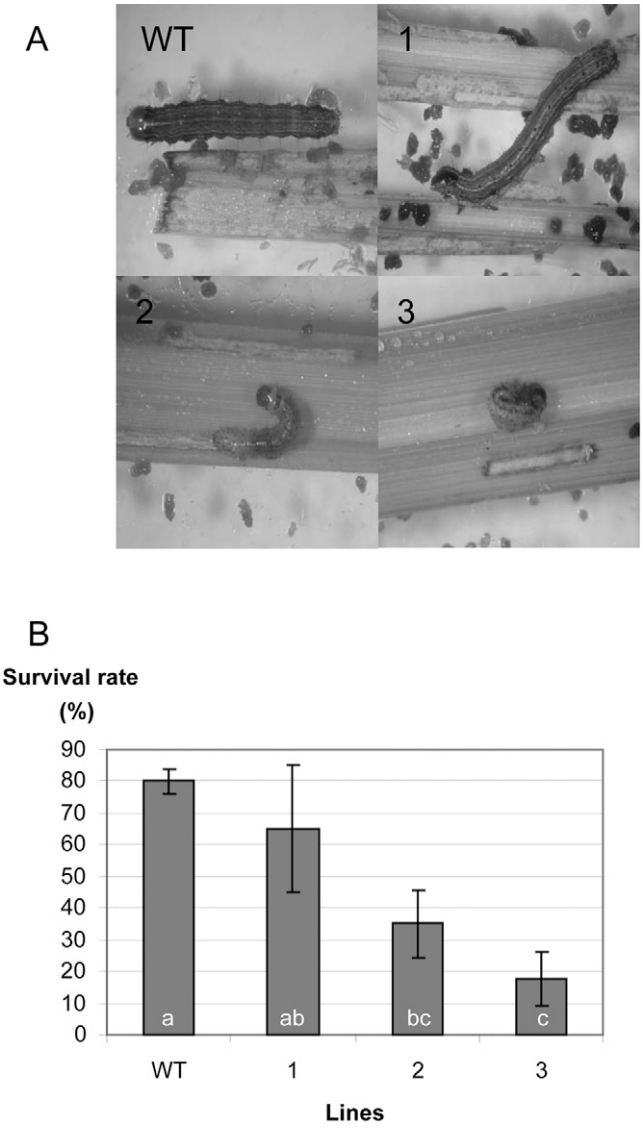

Figure 4. Insect bioassays with fall armyworm neonate larvae feeding on leaves from transgenic bahiagrass lines and wild-type bahiagrass. (A) Feeding pattern of neonate larvae on leaves of transgenic bahiagrass lines (1, 2, and 3) and wild-type (WT) bahiagrass. (B) Survival rate (%) of neonate fall armyworm larvae following feeding on transgenic bahiagrass leaves (lines 1, 2, and 3) and WT bahiagrass. Bars represent standard error. Letters indicate differences at $P < 0.05$ by LSD protected test.

procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19:11–15.

Ferre, J., and J. Van Rie. 2002. Biochemistry and genetics of insect resistance to *Bacillus thuringiensis*. Annu. Rev. Entomol. 47:501–533.

Ferry, N., M.G. Edwards, J. Gatehouse, T. Capell, P. Christou, and A.M.R. Gatehouse. 2006. Transgenic plants for insect pest control: A forward looking scientific perspective. Transgenic Res. 15:13–19.

Fraley, R.T., S.G. Rogers, R.B. Horsch, P.R. Sanders, J.S. Flick, S.P. Adams, M.L. Bittner, L.A. Brand, C.L. Fink, J.S. Fry, G.R. Gallupi, S.B. Goldberg, N.L. Hoffman, and S.C. Wo. 1983. Expression of bacterial genes in plant cells. Proc. Natl. Acad. Sci. USA 80:4803–4807.

Fu, X., L.T. Duc, S. Fontana, B.B. Bong, P. Tinjuangjun, D. Sudhakar, R.M. Twyman, P. Christou, and A. Kohli. 2000. Linear transgene constructs lacking vector backbone sequences generate low-copy-number transgenic plants with simple integration patterns. Transgenic Res. 9:11–19.

Gondo, T., S. Tsuruta, R. Akashi, O. Kawamura, and F. Hoffmann. 2005. Green, herbicide-resistant plants by particle inflow gun-mediated gene transfer to diploid bahiagrass (*Paspalum notatum*). J. Plant Physiol. 162:1367–1375.

Griffitts, J.S., and R.V. Aroian. 2005. Many roads to resistance: How invertebrates adapt to Bt toxins. Bioessays 27:614–624.

James, C. 2005. Global status of commercialized biotech/GM crops. Int. Serv. For the Acquisition of Agri-biotech Applications Briefs No. 34-2005. ISAAA, Ithaca, NY.

Kaur, S. 2006. Molecular approaches for identification and construction of novel insecticidal genes for crop protection. World J. Microbiol. Biotechnol. 22:233–253.

Littell, R.C., G.A. Milliken, W.W. Stroup, and R.D. Wolfinger. 1996. SAS system for mixed models. SAS Inst., Cary, NC.

Meagher, R.L., and R.N. Nagoshi. 2004. Population dynamics and occurrence of Spodoptera frugiperda host strains in southern Florida. Ecol. Entomol. 29:614–620.

Mendelsohn, M., J. Kough, Z. Vaituzis, and K. Matthews. 2003. Are Bt crops safe? Nat. Biotechnol. 21:1003–1009.

Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plant. 15:473–497.

Nagoshi, R.N., and R.L. Meagher. 2004. Behavior and distribution of the two fall armyworm host strains in Florida. Fla. Entomol. 87:440–449.

Nagoshi, R.N., R.L. Meagher, G. Nuessly, and D.G. Hall. 2006. Effects of fall armyworm (Lepidopetera: Noctuidae) interstrain mating in wild populations. Environ. Entomol. 35:562–568.

Odell, J.T., F. Nagy, and N.H. Chua. 1985. Identification of DNA-sequences required for activity of the Cauliflower Mosaic Virus-35s promoter. Nature (London) 313:810–812.

Ranjekar, P.K., A. Patankar, V. Gupta, R. Bhatnagar, J. Bentur, and P.A. Kumar. 2003. Genetic engineering of crop plants for insect resistance. Curr. Sci. 84:321–329.

Rochester, D.E., J.A. Winer, and D.M. Shah. 1986. The structure and expression of maize genes encoding the major heat shock protein, hsp70. EMBO J. 5:451–458.

Romano, A., K. Raemakers, J. Bernardi, R. Visser, and H. Mooibroek. 2003. Transgene organization in potato after particle bombardment-mediated co-transformation using plasmids and gene cassettes. Transgenic Res. 12:461–473.

Sandhu, S., F. Altpeter, and A.R. Blount. 2007. Apomictic bahiagrass expressing the *bar* gene is highly resistant to glufosinate under field conditions. Crop Sci. 47:1691–1697.

SAS Institute. 2002. Proprietary software release 9.1. SAS Inst., Cary, NC.

Schnepf, E., N. Crickmore, J. Van Rie, D. Lereclus, J. Baum, J. Feitelson, D.R. Zeigler, and D.H. Dean. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol. Mol. Biol. Rev. 62:775–806.

Smith, R.L., M.F. Grando, Y.Y. Li, J.C. Seib, and R.G. Shatters. 2002. Transformation of bahiagrass (*Paspalum notatum* Flugge). Plant Cell Rep. 20:1017–1021.

Sparks, A.N. 1979. A review of the biology of the fall armyworm. Fla. Entomol. 62:82–87.

USEPA. 2001. Biopesticide registration action document: *Bacillus thuringiensis* Cry1F protein and the genetic material necessary for its production (plasmid insert PHI8999) in corn plants (006481). Available at http://www.epa.gov/pesticides/biopesticides/product_lists/new_ai_2001.htm (verified 10 Sept. 2007).

Wiatrak, P.J., D.L. Wright, J.J. Morais, and R. Sprenkel. 2004. Corn hybrids for late planting in the southeast. Agron. J. 96:1118–1124.