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Combining *cry1Ac* with QTL alleles from PI 229358 to improve soybean resistance to lepidopteran pests

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Abstract

A QTL conditioning corn earworm resistance in soybean PI 229358 and a synthetic *Bacillus thuringiensis cry1Ac* transgene from the recurrent parent 'Jack-Bt' were pyramided into BC_2F_3 plants by marker-assisted selection. Segregating individuals were genotyped at SSR markers linked to an anitbiosis/antixenosis QTL on linkage group M, and were tested for the presence of *cry1Ac*. Marker-assisted selection was used during and after the two backcrosses to develop a series of BC_2F_3 plants with or without the *crylAc* transgene and the QTL conditioning for resistance BC_2F_3 plants that were homozygous for parental alleles at markers on LG M, and which either had or lacked *cry1Ac*, were assigned to one of four possible genotype classes. These plants were used in no-choice, detached leaf feeding bioassays with corn earworm and soybean looper larvae (Lepidoptera: Noctuidae) to evaluate the relative antibiosis in the different genotype classes. Resistance was measured as larval weight gain and degree of foliage consumption. Few larvae of either species survived on leaves expressing the Cry1Ac protein. Though not as great as the effect of Cry1Ac, the PI 229358-derived LG M QTL also had a detrimental effect on larval weights of both pest species, and on defoliation by corn earworm, but did not reduce defoliation by soybean looper. Weights of soybean looper larvae fed foliage from transgenic plants with the PI-derived QTL were lower than those of larvae fed transgenic tissue with the corresponding Jack chromosomal segment. This work demonstrates the usefulness of SSRs for marker-assisted selection in soybean, and shows that combining transgene-and QTL-mediated resistance to lepidopteran pests may be a viable strategy for insect control.

Abbreviations: CEW – corn earworm, MAS – marker-assisted selection, QTL – quantitative trait locus, SBL – soybean looper, SSR – simple sequence repeat

Introduction

Soybean [*Glycine max* (L.) Merr.] seed yields can be severely reduced under conditions of heavy infestation with lepidopteran pests, especially during early reproductive stages and/or when environmental stresses impede compensatory regrowth (Haile et al. 1998). Endogenous resistance to insect pests has been largely underutilized in soybean, despite attempts to exploit resistance from the insect-resistant plant introductions (PIs) PI 171451, PI 227687, and PI 229358 (Van Duyn et al. 1971; Clark et al. 1972; Hatchett et al. 1976; Kilen et al. 1977). Only three cultivars with host plant resistance (HPR) derived from these PIs have been released, and none of these has been widely accepted by growers due to agronomic inferiority and to the inability of breeders to capture the level of resistance exhibited by the PI ancestor (Boethel 1999). Current integrated pest management programs therefore depend on timely applications of insecticides to reduce yield losses under conditions of heavy infestation.

Advances in biotechnology can facilitate the development of insect-resistant soybean cultivars by means of transformation, genetic mapping, and marker-assisted selection (MAS). MAS can be especially useful for developing insect-resistant genotypes by avoiding the cost and limitations of selection in segregating populations using insect feeding bioassays. MAS also offers the potential to reduce linkage drag and to pyramid genes with similar phenotypic effects into elite genotypes. Towards this end, Rector et al. (1998, 1999, 2000) used restriction fragment length polymorphisms (RFLPs) to map quantitative trait loci (QTLs) associated with corn earworm [*Helicoverpa zea* (Boddie), CEW] resistance in PI 171451, PI 227687, and PI 229358. In a 'Cobb' × PI 229358 population, three CEW antixenosis (i.e., non-preference) QTLs from PI 229358 conditioning resistance were mapped to linkage groups (LGs) D1b, H, and M (Rector et al. 1998). In a later study, Rector et al. (2000) identified three antibiosis QTLs in the same population. These QTLs were located on LG G (resistance QTL from PI 229358), on LG J (resistance QTL from Cobb), and on LG M, in the same genomic location as the antixenosis QTL (resistance QTL from PI 229358).

The feasibility of using MAS in soybean breeding increased substantially with the development of markers based on simple-sequence repeats (SSRs), or microsatellites (Cregan et al. 1999). Merits of SSRs include (1) their abundance and even genomic distribution (Weber and May 1989), (2) their high level of polymorphism (Weber 1990; Maroof et al. 1994), (3) their amenability to amplification with the polymerase chain reaction (PCR), and (4) the ease of detection with semi-automated systems (Ziegle et al. 1992; Rafalski and Tingey 1993; Kresovich et al. 1995). The high level of polymorphism makes them especially valuable in soybean because of the limited genetic diversity among elite North American cultivars and breeding lines (Akkaya et al. 1992; Rongwen et al. 1995). The small amount of DNA needed for PCR is especially important in backcross programs, since young plants can be genotyped and selected prior to flowering. After primer sequences for several hundred SSR markers became available in 1998, Narvel et al. (2001) added SSR markers to the RFLP maps of Rector et al. (1998, 2000) to permit more precise mapping of the resistance QTLs and the identification of SSR markers flanking the QTLs.

Genetic engineering of soybean with heterologous genes conferring HPR also shows promise as a way to increase insect resistance and reduce pesticide applications in soybean (Walker et al. 2000). Stewart et al. (1996) transformed the cultivar Jack with a *cry1Ac* transgene from *Bacillus thuringiensis* which encodes a δ-endotoxin, or crystal (Cry) protein that is toxic to lepidopteran defoliators. Field tests have shown the efficacy of this protein against CEW, velvetbean caterpillar [*Anticarsia gemmatalis* (Hübner)] lesser cornstalk borer [(*Elasmopalpus lignosellus* (Zeller)], and, to a lesser extent, soybean looper [*Pseudoplusia includens* (Walker), SBL] (Walker et al. 2000). The high specificity of each class of Cry protein, however, also limits its insecticidal properties to a subset of the pests associated with a crop. Furthermore, the increasingly widespread use of *cry* genes in transgenic cultivars has raised concerns about the evolution of resistant pest populations (McGaughey and Whalon 1992; Frutos et al. 1999). An additional concern is that some resistant populations exhibit cross-resistance to Cry proteins other than the one(s) to which they have been exposed (Stone et al. 1989; Gould et al. 1992).

Pyramiding multiple *cry* genes has been proposed as one strategy to delay the evolution of resistant pest populations (Tabashnik 1994; Roush 1998), but the aforementioned problem with potential cross-resistance limits the usefulness of certain Cry protein combinations. One alternative gene pyramiding strategy is to pyramid a *cry* gene with another transgene that confers resistance through a completely different mode of action. This strategy was investigated by Santos et al. (1996), who combined the *cry1Ac* transgene with the cowpea trypsin inhibitor gene in *Arabidopsis thaliana*, which is an acceptable host to several soybean lepidopteran pests. Plants with both transgenes were actually damaged more than those with only *cry1Ac*. A possible explanation is that protease inhibitors alter the midgut protease composition. This might interfere with the processing of Cry protoxins into their toxic form and/or accelerate the degradation of the toxic form (Forcada et al. 1996; Keller et al. 1996; Oppert et al. 1997). A third strategy would be to pyramid a *cry* transgene with QTLs which condition for resistance, such as those mapped in PI 229358. This approach could be used to develop cultivars carrying resistance factors with different modes of action (Sachs et al. 1996; Douches et al. 1998). The HPR genes should serve to simultaneously reinforce the resistance of the transgene and extend resistance to some pests that are insensitive to a specific Cry protein.

The objectives of our research were (1) to develop a series of BC_2F_3 plants with various combinations of a *cry1Ac* transgene and the PI 229358 genotype at the QTL on LG M, but with similar genetic backgrounds otherwise, and (2) to evaluate genotypes with or without the transgene, and with or without the PI 229358-derived LG M QTL, for antibiosis against two lepidopteran soybean pests. The LG M QTL was chosen because it explains the greatest amount of resistance of all the QTLs that have been identified in PI 229358 (Rector et al. 1998; Walker et al. 2000); Narvel et al., 2000/2001), and because it conditions both antixenosis and antibiosis to CEW (Rector et al. 1998, 2000).

Materials and methods

Marker-assisted selection

Jack-Bt, a line of 'Jack' engineered with a synthetic *cry1Ac* transgene (Stewart et al. 1996) was used as the recurrent parent in two backcrosses after an initial cross to PI 229358 (Figure 1). MAS began in the BC_1F_1 generation. Eight BC_1F_1 plants were screened with the RFLP probe A584, which is linked to an antixenosis QTL on LG M (Rector et al. 1998). The RFLP protocols used have been described by Lee et al. (1996). A584 was not polymorphic between Jack-Bt and PI 229358, however, and there was not enough time to screen with loosely linked markers prior to crossing, so the second round of backcrosses was done in the absence of information about BC_1F_1 genotypes on LG M.

SSR markers became available in time to genotype the BC_2F_1 generation. We selected those markers which mapped to the region of LG M containing the antixenosis QTL (Cregan et al. 1999). Jack-Bt and PI 229358 were screened first to identify polymorphic SSR loci in the region of interest. SSR markers used to screen this and subsequent generations are shown in Table 1. SSR primers used to genotype the BC_2F_1 generation were labeled with ^{32}P using a T4 polynucleotide kinase labeling protocol (Akkaya et al. 1995) prior to PCR amplification. Most primer sets amplified SSRs with $(ATT)_n/(TAA)_n$ core motifs, though some SSRs with $(AT)_n/(TA)_n$ or $(CT)_n/(TC)_n$ repeats were also amplified. PCR reaction mixes for [^{32}P]-labeled primers contained 20 ng of genomic DNA, 1 mM of Mg^{2+}, 0.15 μM of 3′- and 5′-end primers, 200 μM of each dNTP, 1 × PCR buffer (Perkin Elmer),

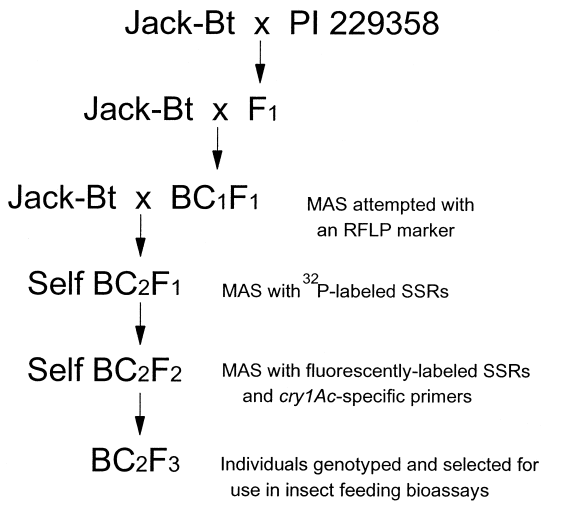

Figure 1. Breeding scheme used to develop BC_2F_3 plants. These plants had various combinations of PI 229358 (resistance) or Jack-Bt (susceptible) alleles at an antixenosis QTL on LG H and at an antibiosis/antixenosis QTL on LG M, and were segregating for the presence of a *cry1Ac* transgene from the recurrent parent, Jack-Bt. Stages at which marker-assisted selection was employed and markers used are indicated.

and 1.25 units of *Taq* DNA polymerase in a 20 μl reaction volume. The PCR cycling program consisted of 32 cycles of (1) 25 s denaturation at 94 °C, (2) 25 s annealing at 47 °C, and (3) 25 s extension at 68 °C for 32 cycles on an ABI 9700 (PE-ABI, Foster City, CA). These cycles were preceded by a 5 min 94 °C denaturation step and were followed by a 3 min final extension step at 68 °C. Radiolabeled amplicons were electrophoresed through 50 cm sequencing gels (5% polyacrylamide, 7 M urea) for 2.5–3.5 h at 60 W in a Sequi-Gen GT System (BioRad, Hercules, CA). Gels were dried and exposed to either X-ray film or a phosphor screen to detect the amplicons.

For genotyping the BC_2F_2 generation, we switched to primers labeled with either blue (6-FAM), green (HEX), or yellow (NED) fluorescent tags (Diwan and Cregan 1997), custom-ordered from PE-ABI (Foster City, CA). Dye-labeled primers for some of the markers used to screen the BC_2F_1 generation were not available, so BC_2F_2 plants were not genotyped at these loci (Table 1). With the switch to fluorescent dye- labeled primers and ABI Prism 377 semi-automated DNA sequencers (PE-ABI), the primer labeling step was omitted and reaction volumes were scaled back to 10 μl. The final extension step in the PCR cycling program was lengthened to 5 min. Samples from three to four different PCR reactions were typically run in each lane. PCR aliquots of 0.4 μl (6-

Table 1. SSR loci on LG M used for marker- assisted selection in three generations. Marker orders are based on the linkage maps of Walker et al. (2000); Cregan et al. (1999). Markers nearest the position to which the LG M QTL was mapped by Walker et al. (2000) are in bold script. Satt markers had an $(ATT)_n$ repeat motif.

Marker	BC_2F_1	BC_2F_2	BC_2F_3
Satt590	–	X	–
Satt540	–	X	X
Satt435	X	X	X
Satt463	X	X	X
Satt245	X	X	X
Satt220	X	–	–
Satt536	–	X	X
Satt175	–	X	X
Satt306	X	X	–

FAM-labeled primers), 0.5 μl (NED-labeled primers), or 0.6 μl (HEX-labeled primers) were pooled in a mixture containing of 2.0 μl formamide, 0.3 μl GENESCAN-500 ROX size standard (PE- ABI), and 0.5 μl of the loading dye supplied with the GENESCAN-500 ROX. Each well was loaded with 0.8 μl (96-well combs) or 1.2 μl (48-well combs) of the pooled sample mixture. Amplicons with different lengths were separated on a 4.8% w/v polyacrylamide gel, with a 12 cm well-to-read distance. Presence of the transgene in each plant was determined using PCR primers specific for the *cry1Ac* sequence, as described by Stewart et al. (1996). Since this is a dominant marker, each DNA sample was screened at least twice to reduce the chances of misclassification.

BC_2F_1 plants (n = 47) were genotyped with the SSR markers listed in Table 1 to determine which lines to advance. A total of 71 BC_2F_2 progeny from two BC_2F_1 plants were genotyped at SSR loci on LG M, and were screened for the presence of the *cry1Ac* transgene. Seeds from selected BC_2F_2 individuals were planted, and 131 BC_2F_3 plants were genotyped at markers flanking the QTL on LG M, and were tested for presence of the *cry1Ac* transgene. The BC_2F_3 plants were also genotyped at three polymorphic SSR loci (Satt442, Sat_122, and Satt541) on LG H which flanked an antixenosis QTL mapped by Rector et al. (1998), and at two SSR loci (Satt228 and Sat_117) flanking an antibiosis QTL mapped on LG G (Rector et al. 2000). This information was used to select genotypes for the bioassays that were unlikely to cause confounding effects on the phenotype.

BC_2F_3 plants with specific genotypes were sorted into four genotype classes based on the presence or absence of the *cry1Ac* transgene and the PI 229358 QTL at SSR markers flanking the QTLs on LG M (Table 2). The eight plants in each class were homozygous at the SSR loci, and either homozygous or hemizygous for the *cry1Ac* transgene. None of the BC_2F_3 plants had alleles from PI 229358 at the SSR loci on LG G, but since the plants were segregating for PI 229358 alleles on LG H, we chose four plants for each class that were homozygous for alleles from one of the parents, and four homozygous for alleles from the other.

Table 2. Genotype classes of plants used in detached leaf bioassays with corn earworm and soybean looper. The predicted genotype at the LG M QTL was determined based on the genotype of flanking SSR markers, and the presence of the *cry1Ac* transgene was detected with sequence-specific primers. Homozygosity for PI 229358 ('229') alleles at SSR markers on LG M is indicated with a 'p' after the linkage group name, and homozygosity for Jack alleles is indicated with a 'j'. Bt+ classes were either hemi-or homozygous for the *cry1Ac* transgene.

Genotype Class	Genotype at LG M QTL	*cry1Ac* transgene
Mp/Bt+	229/229	+
Mp/Bt –	229/229	–
Mj/Bt+	Jack/Jack	+
Mj/Bt –	Jack/Jack	–

Antibiosis bioassay

Antibiosis of BC_2F_3 plants to CEW and SBL was evaluated with no-choice detached leaf bioassays. Entire trifoliolate leaves (if < 12 cm) or 1–2 leaflets from larger trifoliolate leaves were placed in 100 mm × 20 mm petri dishes. Plants were grown in Brown Earth Mix (The Pottery, Commerce, GA) in 5-liter pots, and under a 16 h photoperiod in an insecticide- free greenhouse. An effort was made to keep the amount of leaf tissue as uniform as possible from one dish to another. The petri dishes contained solidified plaster of Paris, ca. 8 mm deep, which was covered with two pieces of 90 mm Whatman No. 1 filter paper (Whatman International, Maidstone, UK) and saturated with dechlorinated tap water to maintain a high ambient humidity within sealed dishes. Excess water was poured off to minimize larval drowning, and leaf samples were infested with three neonate CEW or SBL larvae per plate. The number of SBL larvae per plate was increased from three to five after the first three replications because of a tendency for larvae to wander off of the foliage and die. CEW eggs were obtained from the USDA- ARS Insect Biology and Pop-

ulation Management Laboratory (Tifton, GA), and SBL eggs were obtained from the USDA- ARS Insect Rearing Laboratory (Stoneville, MS). After infestation, the dishes were wrapped with Nescofilm (Karlan Research Products, Santa Rosa, CA) and placed in a walk- in growth chamber. The growth chamber was maintained at 27 °C and 75% ambient humidity, and a 14 h photoperiod was maintained with soft-white fluorescent lights providing ca. 13 μmol photons m^{-2} s^{-1}. CEW larvae were allowed to feed for 5 days and SBL larvae for 7 days.

Data were collected for total larval weight and estimated percent foliage consumption and divided by the number of surviving larvae to obtain mean weight and the percent of foliage consumed by each larva. Leaf consumption percentages were converted to scores ranging from 1 (0–5% per larva) to 7 (30–35% per larva). Multiple samples of leaf tissue (eight for the CEW bioassay and five for the SBL bioassay) were taken from each plant over a 6-week period. For the analysis of variance (ANOVA), individual plants were treated as replications of their particular genotype class, and data for samples from the same plant were used to estimate sampling error. The experiments were analyzed as factorial designs, with two levels for each QTL locus and for the *cry1Ac* transgene.

Since genotyping was conducted concurrently with the bioassays, the plants included in the four genotype classes represent a subset of the 84 BC_2F_3 plants initially included in the bioassays. Eight plants in each genotype class that had complete or nearly complete data sets for all of the samplings were used in the statistical analyses. Jack, Jack-Bt, and PI 229358 plants were also included in the bioassays, but were not included in the statistical analyses because their genetic background differed from that of the backcross genotypes.

The high toxicity of the Cry1Ac protein caused heterogeneity of variances between Cry-positive and Cry-negative plants, so a $\log_{10}(X + 1)$ transformation was used for the larval weight data (Steel and Torrie 1960) prior to ANOVA. Untransformed data were analyzed with Proc GLM in SAS 6.12 (SAS Institute, Cary, NC) to obtain the means for each plant, and transformed larval weight data were analyzed for significant differences between genotypes. For the analysis of the leaf consumption scores using Proc GLM, the mean score for each plant was calculated from the sampling scores, and ANOVA was conducted on the means. Because of the major effect of the *cry1Ac* transgene on all traits, data for the transgenic and nontransgenic classes were also analyzed separately.

In the genotype notation used here, 'Mp' indicates homozygosity for the PI 229358 allele at SSR markers flanking the LG M QTL, and 'Mj' indicates homozygosity for the Jack allele at flanking markers (Table 2). The presence or absence of the *cry1Ac* transgene is indicated as Bt^+ or Bt^-. For example, the plants in the genotype class designated 'Mp/Bt^+' were homozygous for PI 229358 alleles on LG M, and carry the *cry1Ac* transgene.

Results

MAS with SSR markers and a *cry1Ac*- specific primer set permitted the development of BC_2F_3 plants with various combinations of alleles at the antixenosis QTL on LG M and the *cry1Ac* transgene, based on the marker data (Table 2). With two backcrosses, an average of 87.5% of the genomes of these BC_2F_3 plants should have been inherited from Jack-Bt. None of the BC_2F_3 plants had PI 229358 alleles at the SSR loci tested on LG G. The inability to genotype the BC_1F_1 generation at LG M with RFLPs reduced selection efficiency, so most BC_2F_2 plants were still heterozygous at loci in the region of interest. It was therefore necessary to plant a relatively large number of BC_2F_3 plants to ensure recovering several individuals in each of the genotypic classes.

Due to problems with larval survival, even in plates containing nontransgenic Jack foliage, many of the data collected in the bioassays were not suitable for analysis. There were frequently fewer than three larvae recovered from a plate at the end of the feeding period. Decomposed first-instar larval carcasses were often found on the filter paper, regardless of the genotype of the leaf samples in the plate. Since these larvae were impossible to weigh, they were assumed to have had the same weight as neonate larvae (0.2 mg) at the time of death. In other cases, larval carcasses were never found, and high levels of defoliation and/or an unusually large survivor suggested cannibalism, especially in the case of CEW. Data used in the analyses were from sampling dates where larval behavior and survival on the Jack and PI 229358 leaf samples appeared to be normal, and where larval growth and leaf consumption on the experimental genotypes showed a wide range of variation.

Table 3. Significant genotype effects on resistance to corn earworm and soybean looper larvae. Resistance was evaluated based on larval weights and defoliation ratings in no-choice bioassays. Significant main effects and interactions are indicated with the probability of a type I error. Lack of significance is indicated with 'NS'.

Source of variance	Larval weight		Defoliation	
	corn earworm	soybean looper	corn earworm	soybean looper
M	< 0.001	0.01	0.04	NS
Bt	< 0.001	< 0.001	< 0.001	< 0.001
M × Bt	< 0.001	NS	NS	NS

Table 4. Means for main effects in bioassays. Significant difference ($p < 0.05$) associated with either the presence/absence of the Bt *cry1Ac* transgene or the genotype at the LG M QTL locus are indicated by different letters after the numbers within a column. Mean ± SE for parent genotypes (based on eight replications) are shown at the bottom of the table.

Genotype	Larval weight (mg)		Defoliation rating	
	corn earworm	soybean looper	corn earworm	soybean looper
Bt+	0.4 a	2.8 a	1.0 a	1.4 a
Bt –	11.2 b	17.5 b	3.3 b	3.4 b
Mp	4.3 a	7.1 a	2.0 a	2.2 a
Mj	6.9 b	12.3 b	2.3 a	2.6 a
Jack-Bt	0.4 ± 0.2	9.7 ± 3.6	1.1 ± 0.1	2.4 ± 0.7
PI 229358	10.2 ± 3.6	14.8 ± 3.4	2.5 ± 0.3	3.3 ± 0.6

Table 5. Means for genotype classes. Significance difference ($p < 0.05$) between the two classes within the transgenic (Bt+) and nontransgenic (Bt –) groups are indicated with different letters.

Genotype	Larval weight (mg)		Defoliation rating	
	corn earworm	soybean looper	corn earworm	soybean looper
Bt+/Mp	0.3 a	0.9 a	1.0 a	1.1 a
Bt+/Mj	0.5 a	4.9 a	1.0 a	1.6 a
Bt–/Mp	8.6 a	14.6 a	3.0 a	3.2 a
Bt–/Mj	14.0 b	20.3 a	3.6 b	3.5 a

Larval weights

The parental source of the LG M QTL, the presence/absence of the *cry1Ac* gene, and a LG M QTL × Bt interaction all had significant effects ($p < 0.001$) on CEW larval weights (Table 3). Mp was associated with lower larval weights in comparison with Mj. The biggest single factor, however, was the presence or absence of the *cry1Ac* gene (Table 4). A significant LG M × Bt interaction was found for corn earworm larval weight, and was the result of larger differences associated with the effect of the LG M QTL in the absence of the *cry1Ac* transgene (Table 3). No significant LG M × Bt interactions were detected for SBL larval weight or for defoliation by either pest. In the independent analyses of data from the nontransgenic classes, Mp significantly reduced larval weights ($p < 0.01$), whereas in the separate analysis of the transgenic genotype classes, the effect of the genotype at this QTL was not significant (Table 5).

Presence of the *cry1Ac* gene and Mp also reduced the weights of SBL (Tables 3 and 4). When the Bt^+ genotype classes were analyzed independently from the Bt^- classes, there was a significant effect due to the genotype at the LG M QTL ($p < 0.05$). However, in contrast to what was seen with corn earworm, the genotype at the LG M QTL did not contribute significantly to differences between the nontransgenic genotype classes (Table 5).

Leaf consumption

The presence the *cry1Ac* transgene caused a large reduction in defoliation by both pests, and Mp also reduced foliage consumption by CEW (Tables 3 and 4). In the independent analyses of transgenic and nontransgenic data, Mp only reduced CEW defoliation in the absence of Cry1Ac (Table 5).

Analysis of the SBL data showed a highly significant effect from *cry1Ac*, but the effect of the genotype at the LG M QTL was not significant (Tables 3 and 4). The genotype at the LG M QTL did not significantly affect defoliation when the transgenic and nontransgenic classes were analyzed independently (Table 5).

Discussion

Based on the marker data, a *cry1Ac* transgene was successfully pyramided with a QTL for resistance from LG M of PI 229358. Other studies have demonstrated that resistance gene pyramids consisting of a *cry* gene and HPR genes can improve insect resistance in cotton (Sachs et al. 1996) and potato (Douches et al. 1998). In both of these earlier studies, the transgene was expressed in genotypes in which the HPR mechanism and the resulting phenotype were known. Our approach differed in that a resistance allele from a QTL with an unknown mode of action was introgressed into a transgenic line by MAS.

The results of this study show that both the *cry1Ac* transgene and the PI 229358-derived QTL on LG M condition antibiosis to SBL as well as CEW, the pest used to map the QTL (Rector et al. 1998). As SBL weights were significantly lower for the Bt^{-}/Mp class than for the Bt^{+}/Mj class (Table 5), the *cry1Ac* transgene and Mp may have complementary effects against this pest. Both laboratory tests (Stewart et al. 1996) and field tests (Walker et al. 2000) have shown that SBL larvae are less sensitive than CEW and velvetbean caterpillar to Cry1Ac, so the combined effect of the two resistance genes against this pest is promising. The effect of the LG M QTL on resistance to other pests is unknown, as is the mechanism of resistance conditioned by this QTL.

Available marker technology improved continuously during the course of this study, and we modified our methods to take advantage of this, with a progression from RFLPs to radiolabeled SSRs, and finally to fluorescently labeled SSRs. SSR markers and revised maps of the most likely positions of the QTLs are now available (Narvel et al. 2001), which would have made the current work more efficient had they been available for MAS in the BC_1F_1 and BC_2F_1 generations. The number of plants that needed to be genotyped in the later generations could have been substantially reduced. Even if a polymorphic RFLP marker linked to the QTL on LG M had been available, the larger amounts of DNA and time required to use this type of marker instead of SSRs would have limited the number of plants that could have been genotyped prior to flowering.

We focused on antibiosis conditioned by the *cry1Ac* transgene and the PI genotype at the major QTL on LG M. Because of the no-choice nature of the bioassay used here, both leaf consumption and larval weights were considered indicators of antibiosis. The consistent resistance provided by Cry1Ac against the two insect species was expected, as was an effect due to the genotype at the LG M QTL, which explained 37% of the phenotypic variation for defoliation by CEW in a 'Cobb' × PI 229358 population (Rector et al. 1998). First-instar larvae of both pests were very sensitive to the Cry1Ac protein, though evidence from a field study with Jack- Bt suggested that latter instars of SBL are less sensitive (Walker et al. 2000). The data suggest that a single QTL or QTL cluster on LG M conditions resistance to both CEW and SBL. The demonstration of antibiosis towards CEW provides a confirmation of the results of Rector et al. (2000), and shows that the resistance allele on LG M can enhance resistance when transferred from PI 229358 to other genetic backgrounds.

MAS may be the only practical method for pyramiding disease or pest resistance genes that have similar phenotypic effects, especially where one gene masks the presence of other genes. Furthermore, phenotypic selection for insect resistance in early generations of breeding populations is limited by the time and resources needed to conduct bioassays with more than one pest, potential environmental effects (including larval migration), and the inability to plant replications of unique genotypes. The current availability of SSRs and other PCR-based markers linked to QTLs associated with resistance should make MAS a feasible alternative to phenotypic selection for at least some breeding objectives.

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