Restriction Fragment Length Polymorphism Markers Associated with Silk Maysin, Antibiosis to Corn Earworm (Lepidoptera: Noctuidae) Larvae, in a Dent and Sweet Corn Cross

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ABSTRACT Maysin, a C-glycosylflavone in maize silk, has insecticidal activity against corn earworm, *Helicoverpa zea* (Boddie), larvae. Sweet corn, *Zea mays* L., is a vulnerable crop to ear-feeding insects and requires pesticide protection from ear damage. This study was conducted to identify maize chromosome regions associated with silk maysin concentration and eventually to transfer and develop high silk maysin sweet corn lines with marker-assisted selection (MAS). Using an F_2 population derived from SC102 (high maysin dent corn) and B31857 (low maysin *sh2* sweet corn), we detected two major quantitative trait loci (QTL). It was estimated that 25.6% of the silk maysin variance was associated with segregation in the genomic region of *npi286* (flanking to *p1*) on chromosome 1S. We also demonstrated that *a1* on chromosome 3L had major contribution to silk maysin (accounted for 15.7% of the variance). Locus *a1* has a recessive gene action for high maysin with the presence of functional *p1* allele. Markers *umc66a* (near *c2*) and *umc105a* on chromosome 9S also were detected in this analysis with minor contribution. A multiple-locus model, which included *npi286, a1, csu3* (Bin 1.05), *umc245* (Bin 7.05), *agrr21* (Bin 8.09), *umc105a,* and the epistatic interactions *npi286* × *a1*, *a1* × *agrr21*, *csu3* × *umc245*, and *umc105a* × *umc245*, accounted for 76.3% of the total silk maysin variance. Tester crosses showed that at the *a1* locus, SC102 has functional *A1* alleles and B31857 has homozygous recessive *a1* alleles. Individuals of (SC102 × B31857) × B31857 were examined with MAS and plants with *p1* allele from SC102 and homozygous *a1* alleles from B31857 had consistent high silk maysin. Marker-assisted selection seems to be a suitable method to transfer silk maysin to sweet corn lines to reduce pesticide application.

KEY WORDS *Helicoverpa zea, Zea mays, a1-sh2,* favonoid, marker-assisted selection

THE CORN EARWORM, *Helicoverpa zea* (Boddie), is a silk- and kernel-feeding insect that is one of the major pests on corn, *Zea mays* L., in the United States (Dicke and Guthrie 1988). In the southeastern United States, susceptible corn hybrids produce large corn earworm populations that not only cause considerable damage to corn, but adult moths emerging from corn also migrate to other crops for oviposition, which subsequently cause substantial economic losses in cotton, peanut, soybean, and many vegetable crops.

In general, sweet corn germplasm has a low level of resistance to many of the insects and diseases that affect corn (Marshall 1987). Sweet corn is a preferred host and one of the most susceptible crops to the corn earworm (McMillian et al. 1977). Sweet corn is one of the highest ranking vegetable crops in the United States in both market value and total acreage harvested each year, and is also among the top vegetable crops in annual pesticide usage. It is sprayed intensely with insecticides in an effort to totally eliminate insect damage to the ears, which are produced for either the fresh or processed sweet corn markets. The heavy use of synthetic insecticides in agriculture has been of concern to consumers because of food safety, groundwater contamination, the impact on nontarget beneficial organisms, and the environment. Concerns about increased environmental pollution caused by chemical control of plant pests in agriculture are being met with efforts to more fully use the natural defenses of host-plants against these destructive pests. Therefore, the interest in reducing damage of sweet corn by corn earworm larvae using the natural chemical defenses in silks has been revived since the identification of maysin (Guo et al. 1999), which is a C-glycosylflavone (Fig. 1) found in corn silks with antibiotic activity (Widstrom et al. 1977, Waiss et al. 1979). Host-plant resistance to insect feeding on the silks of dent corn can be partially attributed to production of maysin in silks that inhibit corn earworm larval growth

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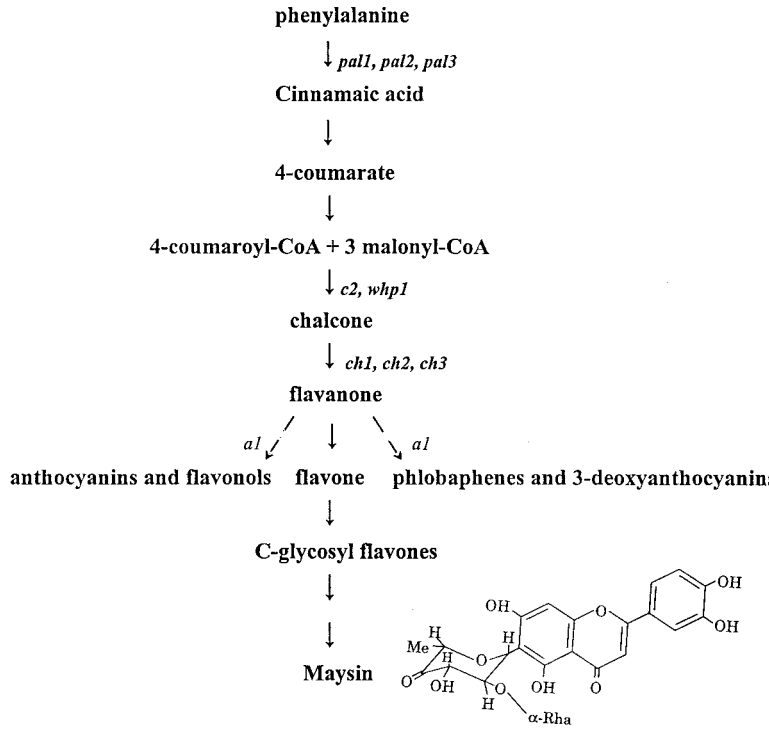

Fig. 1. Flavonoid branch of the phenylpropanoid pathway leading to maysin synthesis in corn. Loci are showed in italics. The pathway before flavanone (*c2, chi* steps) is regulated by *p1* and *r1/c1.* Steps from flavanone to phlobaphenes, 3-deoxyanthocyanins, and C-glycosyl flavones are under control of *p1.* Steps from flavanone to anthocyanins and flavonols are regulated by the *r1/c1* gene family. Chemical structure of maysin, 2″-*O*-α-1-rhamnosyl-6-*C*-(6-deoxy-*xylo*-hexos-4-ulosyl) luteolin, is illustrated.

(Widstrom et al. 1977, Elliger et al. 1980, Wiseman et al. 1996, Widstrom and Snook 1997). An effort to transfer maysin genes to sweet corn has been initiated (Guo et al. 1999).

Maysin synthesis occurs along a branch of the flavonoid metabolic pathway (Fig. 1, Heller and Forkman 1994). In corn and other cereals, two flavonoid biosynthetic pathways have been characterized that are regulated independently by *p1* or *r1/c1* (Styles and Ceska 1989, Grotewold et al. 1994, 1998; Koes et al. 1994). One pathway results in the 3-deoxy flavonoids or C-glycosyl flavones such as phlobaphenes and maysin, whereas the other pathway produces 3-hydroxy flavonoids such as anthocyanins (Styles and Ceska; 1975; Waiss et al. 1979; Snyder and Nicholson 1990; Reid et al. 1992; Grotewold et al. 1994, 1998; Byrne et al. 1996b). Widstrom and Snook (1994) studied the inheritance of silk maysin content in the cross of the dent inbreds GT114 × GT119 and found that a recessive gene was responsible for the high maysin content in the silks of GT114. Using the same cross, Byrne et al. (1996b) conducted quantitative trait loci (QTL) analysis and identified several known flavonoid genes and QTL. A major QTL, the *p1* locus on chromosome 1, had the largest effect on maysin content, and the second major QTL (*umc105a*) on chromosome 9 increased maysin only when this locus was homozygous for the GT119 alleles and there was a functional *p1* allele from GT114 (Byrne et al. 1996b). This recessive gene identified in the cross of GT114 × GT119 may be the same locus and named as *recessive enhancer of maysin1* (*rem1*) (Lee et al. 1998).

C-glycosyl flavones (e.g., maysin) act as insecticidal agents through the subsequent conversion to quinones by polyphenol oxidases. In the larval gut, quinones reduce the availability of free amino acids and proteins by binding to -SH and -NH_2 groups and thus inhibiting larval growth and development (Felton et al. 1989, Wiseman and Carpenter 1995). This oxidization conversion also is responsible for the silk-browning reaction (Levings and Stuber 1971, Byrne et al. 1996a, Guo et al. 1999). Previous laboratory studies showed that the phenotypic correlation coefficient between silk maysin concentration and larval weight had a highly significant negative relationship (Wiseman et al. 1992, Byrne et al. 1997, Guo et al. 1999). Other studies also showed that silk browning was closely related to silk maysin levels (Byrne et al. 1996a, Guo et al. 1999).

Our initial studies of crosses between dent and sweet corn lines suggest that a recessive enhancer gene from sweet corn inbreds, in the homozygous condition and the presence of a functional *p1* allele, increased silk maysin concentration drastically (Guo et al. 1999). In this article, we report the results of a QTL mapping study in the cross of SC102 and B31857,

identification of QTLs that affect silk maysin concentration, and examination of a recessive enhancer gene in the *sh2* sweet corn line B31857 as reported by Guo et al. (1999). Our long-term objective is to identify and examine the QTL associated with silk maysin synthesis and use these QTLs as markers to transfer maysin genes to the commercial sweet corn lines (Guo et al. 1998).

Materials and Methods

Plant Materials. The mapping population was derived from a cross between two corn lines, dent corn SC102 and sweet corn B31857. SC102 was developed at the Pee Dee Experiment Station, Florence, SC, and has high level of maysin in silks. Sweet corn inbred B31857 with *shrunken-2* (*sh2*) endosperm was obtained from Novartis Seeds (Nampa, ID) and has essentially no silk maysin. Based on the phenotypic characters, SC102 has *P1-wrb* alleles (colorless pericarp, red cob, browning silks) at the *p1* locus and B31857 has *p1-www* alleles (colorless pericarp, white cob, nonbrowning silks). We crossed these two parents to an *a1* tester stock and evaluated kernels of the testcrosses for anthocyanins in the aleurone layer. F_1 plants were selfed to produce an F_2 segregating population. The 425 F_2 plants were used in this study for silk collection. Leaf tissue was collected from 286 of these plants for DNA extraction and restriction fragment-length polymorphism (RFLP) genotypic analysis. Backcross individuals of (SC102 × B31857) × B31857 were developed and 200 plants were used in marker-assisted selection (MAS) to test if the F_1 backcross individuals with the markers, which were selected from the RFLP analysis, have consistent high maysin.

Silk Collection and Maysin Phenotypic Analysis. Kernels of parental line, F_1, and F_2 plants were planted in the summer 1998 breeding nursery at the Insect Biology and Population Management Research Laboratory, Tifton, GA. The F_1 backcross kernels were planted in a greenhouse in the winter of 1999. Plants were labeled individually. In preparation for silk collection for maysin analysis, the top ear shoot of each plant was covered with a shoot bag to prevent pollination. Two to three days after silk emergence, the exposed silks were collected from individual plants, placed in a labeled plastic bag, held in a cold box, and transported to the laboratory. Sampled silks were weighed, placed into a 120-ml (4-oz) bottle filled with methanol (100 ml), and then stored at −20°C before chemical analysis by reversed-phase high-performance liquid chromatography (Snook et al. 1989).

Genomic DNA Extraction and RFLP Genotypic Analysis. Genomic DNA was extracted from lyophilized and ground leaf tissue with mixed alkyltrimethyammonium bromide (CTAB) according to the protocol of Gardiner et al. (1993) and Byrne et al. (1996b), except that 4% CTAB was used and 0.5% sodium bisulfite was substituted for β-mercaptoethanol (Hämäläinen et al. 1997). We used 102 probes [90 core markers, Gardiner et al. 1993, and probes *sh2, umc105, p1, php10080, umc55, umc176, glb1, a1, csu392, csu745(rpPo), csu814,* and *npi286* for flavonoid pathway loci or linked to such loci] obtained from the University of Missouri-Columbia. Polymorphism surveys were conducted using single digest with each of the six enzymes (*Eco*RI, *Hin*dIII, *Eco*RV, *Bam*HI, *Dra*I, or *Xba*I) of DNA from the two parents and the F_1. DNA digestion, Southern blotting, and hybridizations were performed as described (Byrne et al. 1996b). Forty-five co-dominant markers were used to survey the F_2 population. Throughout this article, we dissected chromosome into Bin regions and QTL positions referring to the UMC 1998 Maize Working Map published in the *Maize Genetics Newsletter* (Davis et al. 1998).

Statistical Analyses. Segregation at each marker locus was checked for deviation from the expected Mendelian segregation ratio in an F_2 population (1:2:1) by standard chi-square tests. Linkage maps were determined with MAPMAKER/EXP version 3.0 software (Whitehead Institute, Cambridge, MA). Distribution of maysin concentration in the F_2 population was examined with the UNIVARIATE procedure of SAS software (SAS Institute 1989) for deviation from normality. QTLs that affected ($P < 0.05$) maysin concentrations were identified using single factor analysis of variance (ANOVA) (PROC GLM, SAS Institute 1989). Epistatic interactions were identified using two-way ANOVA (PROC GLM, SAS Institute 1989) by evaluating the significance of all possible pairwise combinations of loci. Significant ($P < 0.05$) loci and two-way epistatic interactions were tested in multiple-locus models, from which the "best" model was determined as that which explained the greatest proportion of the phenotypic variance. Significant QTLs also were detected by interval mapping with MAPMAKER/QTL (Whitehead Institute 1993) with the threshold value at LOD > 3.0. Genotypic class means were calculated using the least squares means option (LSMEANS) (PROC GLM, SAS Institute 1989). Differences between genotypic class means were tested using least significant difference (LSD).

Results

The frequency of silk maysin concentrations in the F_2 population exhibited an abnormal distribution with transgressive segregation at the high maysin end (Fig. 2). Significant deviation from normality was observed and transformation was not effective in normalizing the data. We maintained the extreme individual values. About one-third (32%) of the individual F_2 plants had zero or negligible levels of maysin (<0.05% fresh silk weight) and 26% had silk maysin concentrations varying from 0.05 to 0.2%. Twenty-eight percent of the plants had maysin levels >0.2% but <0.6%. However, 14% of the individuals contained >0.6% silk maysin, which exceeded the maysin level of the high maysin parental line SC102 (0.58%) (Fig. 2). The means for maysin concentrations in the F_1 and F_2 were similar. These data suggest that maysin biosynthesis in this cross may be primarily under additive gene control in the inheritance of silk maysin concentration.

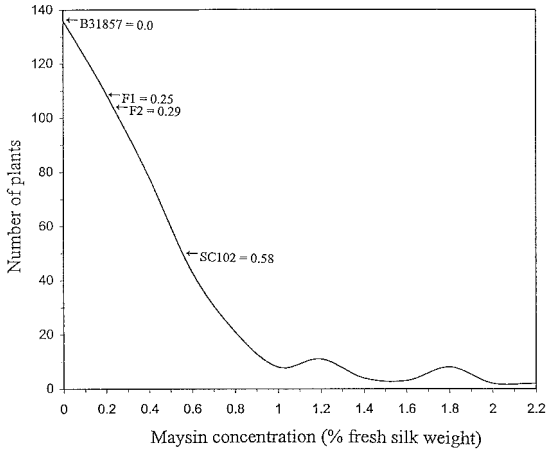

Fig. 2. Frequency distributions of silk maysin concentration in a (SC102 × B31857) F_2 population. Means of parents, F_1, and F_2 are indicated with arrows.

Table 2. Multiple-locus model of QTLs and epistatic interactions that explain variance for silk maysin concentration in the population (SC102 × B31857)F_2

Locus or interaction	Bin[a]	Significance (P^b)
npi286 (near *p1*)	1.03	0.0001
csu3 (near *p1*)	1.05	0.0001
a1	3.09	0.0001
umc245	7.05	0.0002
agrr21	8.09	0.0002
umc105a	9.02	0.031
npi286 × *a1*		0.0001
csu3 × *umc245*		0.0001
a1 × *agrr21*		0.003
umc105a × *umc245*		0.03
Total R^2 = 76.3%		
n = 266		

[a] Dissection of chromosome into Bin regions and QTL positions refer to the UMC 1998 Maize Map published in the Maize Genetics Newsletter 72 (Davis et al. 1998).

[b] P value from the analysis of variance F test. Loci or interactions significant at $P < 0.05$ were retained in the model which explained 76.3% phenotypic variance.

Test crosses for the *a1* locus indicated that the SC102 genotype is *A1/A1* alleles and that B31857 is *a1/a1* alleles. Segregation ratios were significantly distorted ($P < 0.01$) from the expected Mendelian segregation (1:2:1) for seven markers: *asg62* (Bin 1.07), *umc34* (Bin 2.04), *php20608* (Bin 4.10), *umc90* (Bin 5.02), *umc85* (Bin 6.01), *umc245* (Bin 7.05), and *bnl2.369* (Bin 8.05).

Six QTLs on four chromosomes were detected for silk maysin concentration using single-factor ANOVA (Table 1). Silk maysin concentration was significantly ($P < 0.05$) associated with chromosome 1 (*npi286* Bin 1.03, *csu3* Bin 1.05, and *umc67* Bin 1.06), chromosome 3 (*a1* Bin 3.09), chromosome 4 (*umc66a* Bin 4.07), and chromosome 9 (*umc105a* Bin 9.02). MAPMAKER/QTL analyses identified that two positions with the peak LOD scores were the previous map position (Davis et al. 1998) of the *p1* locus (LOD = 21.4) on chromosome 1 and *c2* locus (LOD = 20) on chromosome 4. Two other QTL locations identified on chromosome 1 were between *umc67* and *asg62* (LOD = 13.5) in the centromere region and *umc128*(aga) (Bin 1.07, LOD = 11.2) on the long arm.

All alleles contributing high silk maysin concentration originated from the high maysin parent SC102, except for the loci *a1* and *umc66a* on chromosomes 3 and 4, respectively. The six QTLs that had significant effects on maysin concentration accounted for 2–26% of the total phenotypic variance, respectively (Table 1). The significant effect ($P < 0.0001$) of *npi286* and *csu3* on chromosome 1 in the single-factor analysis is most likely due to linkage with the *p1* locus (Table 1). Therefore, the *p1* and *a1* regions were the locations with the largest effect on silk maysin concentration in this population (Table 1).

In the multiple-locus model, we identified six individual loci on five chromosomes and four epistatic interactions that had significant ($P < 0.05$) effects on silk maysin concentration (Table 2). Two loci, *umc245* (Bin 7.05) and *agrr21* (Bin 8.09), were retained in this model, but, were not significant in the single-factor analysis (Table 1). The markers *umc67* and *umc66a* were significant in the single-factor analysis (Table 1) but not included in this multiple-locus model (Table 2). Marker *umc105a* on chromosome 9 was significant only at $P = 0.031$. The epistatic interactions between *npi286* (*p1*) and *a1*, *csu3* (*p1*) and *umc245* (chromosome 7) were significant at $P < 0.0001$. The multiple-locus model explained 76.3% of the total phenotypic variance (Table 2).

Genotype class means of silk maysin concentration for individual loci were significantly different ($P <$

Table 1. Significant loci in the single-factor ANOVA for silk maysin concentration in the population (SC102 × B31857)F_2

Locus	Bin[a]	Significance (P^b)	R^2 (%)[c]	Parent contributing high value allele
npi286 (near *p1*)	1.03	0.0001	25.6	SC102
csu3 (near *p1*)	1.05	0.0001	17.9	SC102
umc67	1.06	0.0001	7.0	SC102
a1	3.09	0.0001	15.7	B31857
umc66a (near *c2*)	4.07	0.007	2.8	B31857
umc105a	9.02	0.01	2.3	SC102

[a] Dissection of chromosome into Bin regions and QTL positions refer to the UMC 1998 Maize Map published in the Maize Genetics Newsletter 72 (Davis et al. 1998).

[b] P value from the analysis of variance F test.

[c] Percent phenotypic variance explained.

Table 3. Genotype class means for silk maysin concentration in (SC102 × B31857)F_2

Locus[a]	Genotype[b]	Bin[c]	Mean maysin concn (% fresh silk wt)[d]
npi286 (near *p1*)	A	1.03	0.552a
	H		0.282b
	B		0.035c
csu3 (near *p1*)	A	1.05	0.520a
	H		0.252b
	B		0.102c
umc67	A	1.06	0.481a
	H		0.245b
	B		0.222b
a1	A	3.09	0.207a
	H		0.213a
	B		0.717b
umc66a (near *c2*)	A	4.07	0.233a
	H		0.290a
	B		0.387b
umc105a (near *bp1*)	A	9.02	0.448a
	H		0.267b
	B		0.263b

[a] Loci selected if significant at $P < 0.01$ in the single-factor analysis.
[b] Genotype A, H, B represent homozygote for the allele from parent SC102 dent corn, heterozygotes (one SC102 allele and one B31857 allele), and homozygote for the allele from parent B31857 sweet corn, respectively.
[c] Dissection of chromosome into Bin regions and QTL positions refer to the UMC 1998 Maize Map published in the Maize Genetics Newsletter 72 (Davis et al. 1998).
[d] Within each locus, means followed by the same letter are not significantly different at the 0.05 probability level.

0.05) at all loci (Table 3). The gene action of the major QTL at the *p1* region (*npi286* and *csu3* as linked markers) was strikingly additive. The genotype class means suggest that *p1* region have additive gene action for high maysin. The genotype class mean of epistatic interaction for maysin indicates that the gene action at the *a1* region increases maysin concentration significantly only when *a1* alleles from B31857 are homozygous and a functional *p1* allele from SC102 (A or H class) is present (Table 4). Therefore, the gene action at the *a1* region is dominant for low maysin and is only expressed when the pathway is activated by *p1*.

Table 4. Genotype class means for the *npi286* × *a1* epistatic interaction for silk maysin concentration in (SC102 × B31857)F_2

Genotype[a]		Fresh silk wt (%)
npi286 (near *p1*)	*a1*	Mean maysin concn
A	A	0.376a[b]
A	H	0.382a
A	B	1.276b
H	A	0.153c
H	H	0.196c
H	B	0.729d
B	A	0.001f
B	H	0.049f
B	B	0.025f

[a] Genotype A, H, B represent homozygote for the allele from parent SC102 dent corn, heterozygotes (one SC102 allele and one B31857 allele), and homozygote for the allele from parent B31857 sweet corn, respectively.
[b] Within each genotypic interaction, means followed by the same letter are not significantly different at the 0.05 probability level.

Based on the RFLP analysis and the results as described above, we used *npi286* as flanking marker to *p1*, and *a1* in MAS to examine 200 F_1 backcross plants. Individuals with genotypes H at *npi 286* (*p1*, heterozygotes for the alleles from each parental SC102 and B31857) and B at *a1* (homozygotes for the alleles from parental B31857) were selected with maysin level averaged at 0.39% of fresh silk weight, which was higher than the averaged maysin levels of F_1 (0.25%) and F_2 (0.29%).

Discussion

To develop sweet corn inbreds and hybrids with protective levels of maysin in silks by trait transfer with DNA-marker assistance, we conducted a genetic analysis in a F_2 segregating population derived from the high maysin dent corn inbred SC102 and a non-maysin *sh2*-sweet corn inbred B31857. Two major QTL have been identified using single-factor ANOVA, *p1* (*npi286* and *csu3* as flanking markers) on the short arm of chromosome 1 ($R^2 = 25.6\%$) and *a1* on the long arm of chromosome 3 ($R^2 = 15.7\%$). Two minor QTLs also were detected by this analysis, *umc66a* (near *c2*) on the long arm of chromosome 4 and *umc105a* (*brown pericarp1, bp1*) on the short arm of chromosome 9 (Meyers 1927, Emerson et al. 1935, Byrne et al. 1996b), also designated as *rem1* by Lee et al. 1998. Two other QTLs also were detected by MAPMAKER/QTL in the centromere region (interval of *umc67* and *asg62*) and on the long arm (*umc128* Bin 1.07) of chromosome 1, respectively. The multiple-locus model analysis retained marker *umc245* on the long arm of chromosome 7, *agrr21* on the long arm of chromosome 8, and the epistatic interaction *p1* × *a1*, *p1* × *umc245*, *a1* × *agrr21*, and *umc105a* × *agrr21*. Our results are in agreement with the QTL analyses by Byrne et al. (1996b 1998) and Lee et al. (1998), in general. Our study first demonstrated the major contribution of *a1* to silk maysin biosynthesis.

To study insect resistance in sweet corn germplasm, Robertson and Walter (1963) initiated a translocation linkage study in an attempt to locate genes that might be responsible for the resistance to the corn earworm in the sweet corn lines. Ten years later, Widstrom and Wiseman (1973) carried out a similar study, intended as an extension of Robertson and Walter (1963), that involved six sweet-corn inbreds. Both studies had one sweet corn in common and both located a gene on chromosome 5. However, Widstrom and Wiseman (1973) determined another gene on the long arm of chromosome 4 for resistance in the same sweet corn inbred line. In our current study, we detected two QTLs from sweet corn inbred B31857 on chromosome 3 (*a1*) and four (*umc66a*, near *c2*) that, with appropriate complementary factors carried over by SC102 (e.g., presence of the functional *p1* allele), significantly contribute to the transgressive segregation in the F_2 progeny with high silk maysin concentration.

We again demonstrated a fundamental role of the major regulatory locus *p1* in controlling the flavone pathway branch and synthesis of maysin (Byrne et al.

1996b, 1998). Recently, Grotewold et al. (1998) demonstrated the induction of specific flavonoids and phenylpropanoids by ectopic expression of transgenes encoding regulatory transcription factors (C1/R or P) in cultured black Mexican sweet corn cells. The predominant compounds induced by expression of *p1* (encoding P protein) are C-glycosyl flavones closely related to maysin in silks. Additional support for *p1* regulatory role is its additive QTL gene action and its interaction with other loci (Byrne et al. 1996b, Grotewold et al. 1998).

The second maysin QTL is *a1*, which shows dominant gene action for low maysin and significant epistatic gene action with *p1*, and may be the recessive maysin enhancer gene from *sh2* sweet corn as reported by Guo et al. (1999). The dominant functional allele *A1* causes anthocyanin pigments to form in the aleurone, plant, and pericarp tissues; the recessive nonfunctional *a1* allele causes absence of pigment (colorless) in these tissues (Neuffer et al. 1997). The *A1* encodes for a dihydroflavonol 4-reductase (Reddy et al. 1987, Schwarz-Sommer et al. 1987, Bernhardt et al. 1998). This enzyme is involved in two branches of the flavonoid pathway (Fig. 1) and converts dihydroflavonols as well as flavanones (Styles and Ceska 1975, 1989). Dihydroflavonols are the precursors of 3-hydroxyanthocyanins, whereas flavanones are precursors of the 3-deoxyanthocyanins and phlobaphenes (Fig. 1). Styles and Ceska (1977) reported that the *A1* allele is required in the pathways of both the *c1/b1* controlled anthocyanins and the *p1* regulated 3-deoxyanthocyanins, but not in the pathway of *p1* regulated flavones (Fig. 1). Homozygous recessive *a1* alleles would block the pathways and prohibit the synthesis of the 3-hydroxyanthocyanins and 3-deoxyanthocyanins, resulting in the release of precursors or intermediates for the *p1* regulated flavone pathway (Styles and Ceska 1975, 1989). Therefore, a block at the *a1* locus could enhance C-glycosyl flavone biosynthesis and increase or enhance the end products (e.g., maysin) as demonstrated by a recent study (Pelletier et al. 1999), using *Arabidopsis* as a model for studying the flavonoid biosynthetic pathway regulation. Pelletier et al. (1999) revealed that mutant lines blocked at intermediate steps of the pathway actually accumulated higher levels of specific flavonoid enzymes and other end products. The *p1* × *a1* epistatic interaction in this study indicates the shifting of precursors or intermediates to the flavone pathway and results in high silk maysin, which agrees with a previous study (Styles and Ceska 1989). Styles and Ceska (1989) reported that in the presence of functional *p1* the level of flavones in developing pericarp tissue is enhanced by the homozygous recessive *a1.* This may be due to a shifting of intermediates to the flavone pathway (Pelletier et al. 1999).

We detected a QTL (near *c2*) on the long arm of chromosome 4 that had a significant effect on maysin concentration ($P < 0.007$, $R^2 = 2.8\%$). The *c2* locus encodes chalcone synthase (Dooner 1983, Dooner et al. 1991, Franken et al. 1991), a key enzyme for synthesis of all flavonoid compounds (Fig. 1) and a rate-limiting enzyme. This locus may be the chromosomal location associated with the resistance to corn earworm in the sweet corn line tested by Widstrom and Wiseman (1973).

Two QTLs (*umc245* and *agrr21*) on the long arms of both chromosome 7 and 8 were retained in the multiple-locus analysis, which had a significant ($P < 0.003$) epistatic interactions with *csu3* (*p1*) and *a1*, respectively. Byrne et al. (1998) reported a QTL on the long arm of chromosome 8 for maysin concentration. N.W.W. and M.E.S. (unpublished data) reported the translocation studies on chromosome locations of major genes controlling biosynthesis of silk maysin and its analogues and compared their study with previous QTL studies. They found that all chromosomes, except 7, have been associated to some degree with flavone synthesis. This QTL on chromosome 7 may be a previously undetected position and more study is needed to characterize this new QTL.

Our previous laboratory bioassay using lyophilized dry-silk of the F_2 populations derived from crosses between high maysin dent corn inbred lines and low maysin *sh2*-sweet corn inbred lines produced low corn earworm larval weights as a result of the high maysin concentration in silks (Guo et al. 1999). This study provides excellent promise that damage to sweet corn by corn earworm could be reduced by increasing silk maysin concentration. Phenotypic correlations between maysin concentrations and larval weights were negative. Waiss et al. (1981) showed that 0.15% maysin reduced corn earworm larval weights by 50%. Wiseman et al. (1992) suggested that 0.2% silk maysin decreased corn earworm larval weight by 50%. In another study, Snook et al. (1993) indicated that 0.2% maysin level in silks was necessary for effective resistance to corn earworm. In this study, our maysin data showed that the average levels of maysin in silks of F_1s and F_2s were 0.25 and 0.29%, respectively. The F_1 backcross individuals with heterozygous *p1* from SC102 and homozygous *a1* from B31857 had maysin level averaged at 0.39%, which exceed the 0.2% threshold level suggested by Snook et al. (1993) and Wiseman et al. (1992).

Production of sweet corn with undamaged ears depends heavily on pesticides to reduce ear damage caused by the corn earworm. In this study, we identified at or near QTL *p1* and *a1* with substantial effects on silk maysin levels. The tight linkage between *a1* and *sh2* (i.e., 0.2cM apart, Neuffer et al. 1997) suggests that *a1* was concurrently introgressed with *sh2* into the progeny as suggested by Guo et al. (1999) who noted that phenotype plants from sweet corn kernels in the dent and *sh2*-sweet corn crosses always have higher silk maysin, if maysin is present, than plants from dent kernels. In our unpublished study, we used markers *p1*, *a1*, and *sh2* to probe the F_1 backcross progeny of the same cross material, and selected and backcrossed the individual progenies with the heterozygous *p1* allele and homozygous *a1* and *sh2* alleles to the recurrent parental line, in which the maysin analysis showed that these progenies had the highest maysin levels (unpublished data). Our silk bioassay study (Guo et al.

1999) provides excellent promise that damage to sweet corn by corn earworm can be reduced by increasing silk maysin concentration and also shows that it should not be difficult to select for and transfer maysin to sweet corn lines developed from this cross. While selecting for high maysin alleles from dent corn lines, we should pay close attention to select and maintain sweet corn quality factors. Further confirmation and application of marker-assisted transfer of maysin genes will help our understanding and will also help in breeding sweet corn lines with high antibiosis to ear-feeding insects such as corn earworm. Conversion of RFLP-based markers into polymerase chain reaction-based markers should be considered for practical reasons.

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