Maize silk antibiotic polyphenol compounds and molecular genetic improvement of resistance to corn earworm (*Helicoverpa zea* Boddie) in *sh2* sweet corn

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Abstract

The flavor of *sh2* super-sweet corn is preferred by consumers. Unfortunately, *sh2* sweet corn has little genetic variation for insect resistance. In this paper we review the functions of two loci, *p1* and *a1*. The *P1* allele has a major role in *sh2* sweet corn resistance to corn earworm, an allele that was lost in historical selection because of its pleiotropic effect on undesirable cob color and silk browning. The *P1* allele has significant effects on biosyntheses of silk antibiotic compounds, maysin, apimaysin, methoxymaysin, and chlorogenic acid. The effect of *a1* shows gene action for lowered maysin and significant epistatic action with *p1*. The dominant functional allele *A1* causes anthocyanin pigments in aleurone, plant, and pericarp tissues; the recessive *a1* allele causes absence of pigment in these tissues. If silk browning and cob color are critical factors for maysin production but lack the customer's preference, then separating red cob and browning silk, which are controlled by the *P1* allele, may be difficult if not impossible. One high silk maysin *sh2* sweet corn germplasm, *shrunken* Zapalote Chico, has been released. There is some field corn germplasm with *p1-wwr* alleles, but the amount of antibiotic flavones and their potential as a donor need further investigation.

Introduction

The corn earworm (*Helicoverpa zea* Boddie) attacks several crops worldwide.[1-3] This pest was classified by Wiseman and Davis[4] as the most destructive maize (*Zea mays* L.) pest in the Southeastern United States. Economic damage in maize is caused by consumption of the kernels by larvae and exposure of the ear to possible microbial infection, resulting in mycotoxin contamination of the maize crop.[5] Wiseman[6] stated that females lay eggs on fresh silks and neonate larvae move from exposed fresh silks to a more protected position in the silk channel formed by a husk extension. Larvae begin to feed on silks and, when silks do not supply enough food or husks are sufficiently loose, move through the silk channel and feed on kernels. Therefore, the presence of silk antibiotic compounds or structural characteristics, such as a long silk channel or good husk coverage, which make access to grain difficult,[7-9] is desirable to prevent ear damage by corn earworm larvae. However, several authors have determined that husk protection is affected greatly by moisture stress and does not give consistent protection over years and in different locations.[10-12]

Sweet corn is one of the most popular and economically important vegetables in the United States. Laughnan[13] suggested that the *shrunken2* (*sh2*) allele may have an application in the sweet corn industry, resulting in *sh2* sweet corn that is preferred by consumers for flavor.[14] The genetic base of sweet corn is largely from the northern flints,[15] which tend to be more susceptible to many pests than indigenous North American races of corn. Because of the adaptation to an area where winter temperatures are generally too low to allow pupation of the corn earworm larvae, thus resulting in minor infestations, there is little selection for resistance to this pest. Breeding efforts made to extend the range of sweet corn southward involved crosses of the northern varieties with the southern dents,[16,17] which resulted in varieties with adaptation to the Southern United States and resistance to corn earworm. Mechanical resistance to severe corn earworm damage is a result of longer and tighter husks,[14,17] which are not preferred in mechanized sweet corn production.[14] Consequently, because of the consumer's zero tolerance for sweet corn ear damage in US markets, sweet corn growers in the Southern United States spray insecticide as many as 25 to 40 times per season to prevent insect damage to ears.[18,19] This extensive use of synthetic insecticides in agriculture is of concern because of worker and consumer safety and environmental contamination.

Silk browning and cob color are controlled by the *p1* gene, which also regulates pericarp color.[20-22] In sweet corn, the preferred cob color is white. In the development of sweet corn, it was noted that silk browning was associated with undesirable cob color[23] and this characteristic proved to be a convenient selection criterion to exclude the undesirable cob color and browning silks. The silk browning was later found to be associated with compounds in the phenylpropanoid pathway (Figure 1), which have an antibiotic effect against the corn earworm.[19,24] Among the natural products synthesized through this pathway are C-glycosyl flavones, including maysin, apimaysin and methoxymaysin, and the phenylpropanoid compound chlorogenic acid, which are found in corn silks.[25-27] On silk wounding by an insect, flavones and phenylpropanoids are oxidized by polyphenol oxidase to quinines, which can bind to the –SH and –NH^2 groups of free amino acids and proteins, making them unavailable for the insect growth,[28] and resulting in silk browning.[19,20,23] Because the *P1* allele is associated with undesirable traits, colored cob and silk browning, this allele was selected against during development of sweet corn and lost in the breeding selection process.

Maysin concentration in maize silks is controlled genetically by well characterized flavonoid pathway genes such as *p1* and by quantitative trait loci (QTLs) including some relatively poorly understood loci as revealed by

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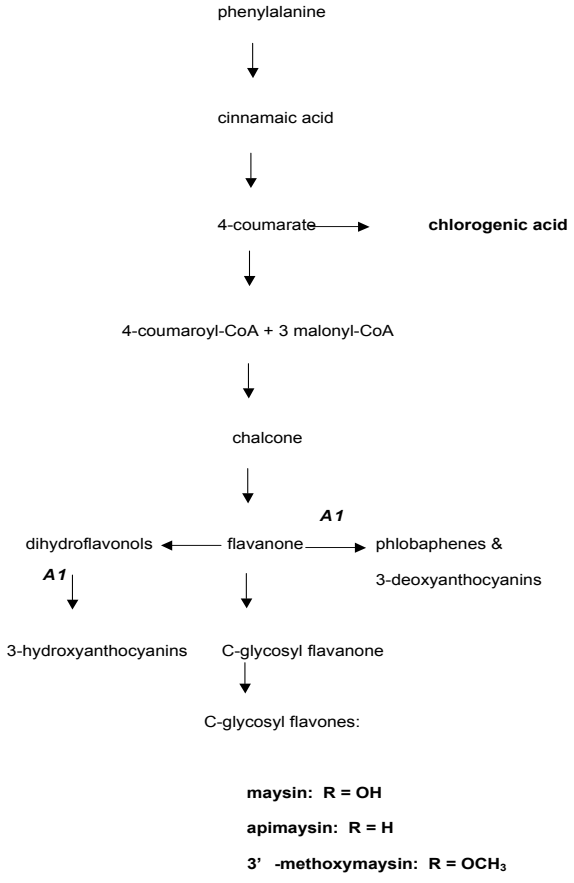

Figure 1. A schematic part of the flavonoid and phenylpropanoid pathways (adopted from Guo *et al.*, 2004) leading to antibiotic compound biosynthesis in maize silks: intermediates, competing branch pathways, and the structures of chlorogenic acid and C-glycosyl flavones including maysin, apimaysin, and methoxymaysin. Functional *A1* allele of *a1* gene encodes for a dihydroflavonol 4-reductase (Bernhardt *et al.*, 1998), which is involved in two branches of the pathway.

DNA markers.[29-34] In QTL studies, the *p1* gene is always identified within a major QTL region controlling maysin concentration.[31,33-36] The functional *P1* allele encodes a Myb-homologous protein that can bind to and activate transcription of the *a1* gene, and regulates the transcription of other flavonoid pathway genes.[32,37] The *p1* gene dictates the level of downstream biosynthetic gene expression and acts in an additive fashion. The functional *A1* allele encodes an enzyme that is involved in two branches of the flavonoid pathway, and converts dihydroflavonols and flavanones[30] to 3-hydroxyanthocyanins, 3-deoxyanthocyanins, and phlobaphenes (Figure 1).

Apimaysin and methoxymaysin are highly related to maysin, structurally differing only by a 3'-hydroxyl group (apimaysin 3'-H, methoxymaysin 3'-OCH_3, maysin 3'-OH). It had been suggested that apimaysin and maysin share the same structural enzymes,[38] except flavonoid 3'-hydroxylase, and require the same pools of metabolic precursors. Instead, Lee *et al.*[38] suggested that the synthesis of apimaysin and maysin occurs independently, on the basis that an apimaysin QTL did not affect maysin synthesis and a maysin QTL did not affect apimaysin synthesis. Chlorogenic acid synthesis is not well understood nor is the genetic mechanism underlying its concentration in maize silk. In cultured maize cells, Grotewold *et al.*[32] observed a compound that was indistinguishable from chlorogenic acid in the UV absorption spectrum. This compound accumulated when *p*1 gene was expressed, suggesting that *p1* gene expression can affect the level of chlorogenic acid.

We have characterized maize silk antibiosis to corn earworm and the antibiotic compounds in populations derived from four *sh2* sweet corn lines crossed to two field corn lines.[19] Transgressive segregation at the high maysin end was observed in all four populations derived from these crosses. Guo *et al.*[19] suggested that a recessive enhancer from sweet corn in combination with a dominant factor from the field corn resulted in drastically high maysin concentration. This motivated us to map these two factors in the genome[33,34] to identify markers associated with silk antibiosis. In this review, we summarized the quantitative genetic control (QTLs) of synthesis of not only maysin, but also AM-maysin (apimaysin and methoxymaysin), and chlorogenic acid.[34] Specifically, we reviewed the genetic variation and regulation of silk antibiotic compounds, and whether *sh2* sweet corn combination of alleles of *P1* and *a1* from different parental lines would have increased concentrations of these compounds and the utilization of these as markers in marker-assisted selection in *sh2* sweet corn breeding and improvement.

Antibiotic compounds in maize silks

Since Waiss *et al.*[25] identified a C-glycosyl flavone named maysin in maize silks that inhibits growth of corn earworm larvae, many studies have been conducted on its molecular characterization, variability of concentration over genotypes, and determination of its biological activity and inheritance. Widstrom *et al.*[39] summarized the detailed identification, biosynthesis pathway, and the toxicity to corn earworm in the laboratory bioassay. Waiss *et al.*[40] showed that 0.15% of maysin in fresh silk weight reduced larval weight by 50%. Wiseman *et al.*[41] observed that maysin concentration of 0.2% fresh silk weight reduces larvae weight to less than 50% and levels of maysin higher than 0.4% reduce weight of larvae by more than 75% in comparison with the control. Snook *et al.*[42,43] proposed a threshold necessary for effective resistance to corn earworm of 0.2% fresh silk weight.

Although maysin has been identified as the predominant factor for antibiosis to corn earworm in corn silks, Elliger *et al.*[26,27] and Snook *et al.*[44,45] identified several analogues of maysin with antibiotic activity against corn earworm larvae. These authors compared the activity of maysin with that of other antibiotic compounds such as chlorogenic acid, 3'-methoxymaysin, apimaysin, isoorientin, and C-4''-hydroxymaysin and found that their activities ranged from half to equal that of maysin, depending on the number of OH groups at the 3' and 4' positions of the flavonoid B ring. Snook *et al.*[45] and Wiseman *et al.*[41] indicated that these allelochemicals could be a major factor for corn earworm resistance if their concentrations in the silks were as high as the concentrations of maysin in resistant genotypes. Therefore, Widstrom *et al.*[39] concluded that total antibiotic effect of silk chemicals against *H. zea* could depend on the total concentration of all active polyphenols.

Morphological and molecular markers for silk antibiotic compounds

Levings and Stuber[20] reported that silk browning, caused by wounding the silks and subsequent oxidation of dihydroxyl flavones, was controlled by a single dominant gene. Felton and co-workers[46,47] found that, when leaf tissue was damaged by insect feeding, polyphenol oxidases and orthohydroxyphenolic substrates come into contact resulting in the rapid oxidation of phenolics to orthoquinones, which bind or alkylate to -SH and -NH_2 groups of free amino acids and proteins in the larval gut, reducing their availability and inhibiting larval growth and development. Wiseman and Carpenter,[28] trying to explain the action mode of the growth inhibiting factor present in "Zapalote Chico" silks, found that it was not a feeding deterrent or less protein in the diet but an antinutritive factor, such as that reported by Felton *et al.*[46] In more recent studies, a close relationship was found between the silk-browning reaction and silk maysin or related compound levels[19,24] because, on wounding of silk tissue, maysin and related compounds are believed to be oxidized to quinones that are responsible for the silk-browning reaction.[24] Therefore, the silk-browning reaction could be utilized as a promising morphological marker to detect high concentrations of antibiotic compounds in silks.

Syntheses of antibiotic flavonoid compounds occur via a branch of the phenylpropanoid/flavonoid pathway.[48,49] Some of the steps in this pathway have been characterized genetically and biochemically.[30,50,51] Based on the current understanding of the flavonoid pathway, C-glycosyl flavone synthesis requires appropriate alleles at *p1*, a transcriptional regulator, and at the structural genes *c2* or *whp1*, one of *chi* genes, *pr1* and/or other genes controlling the 3'-hydroxylation of the flavonoid B ring, and unidentified additional loci encoding flavone synthase, C-glycosyl transferase, glucose oxidase, rhamnosyl transferase, and an enzyme for transport to the vacuole.[50]

Styles and Ceska[29] pointed out that branches of the flavonoid pathway leading to synthesis of C-glycosyl flavones, phlobaphenes, and 3-deoxyanthocyanins were regulated by the *p1* locus, and Coe and Han[21] found that some allelic variants of *p1* locus were also responsible for the silk-browning reaction. In addition, Grote - wold *et al*.[32] found that *P* expression can affect the levels of specific phenylpropanoids such as chlorogenic acid, since C-glycosyl flavones were the predominant compounds induced by ectopic expression of the transgene encoding the regulatory transcription factor *p1*, but increased levels of specific phenylpropanoids were also induced. Genes *c2* and *whp1* encode chalcone synthase, which catalyzes an early step in the flavonoid pathway.[52] The next step in the pathway is controlled by one *chi* gene that encodes chalcone isomerase.[53] Locus *p1* regulates the accumulation of chalcone synthase (C2) and chalcone isomerase (CHI).[30,54] Since several steps in the synthesis of polyphenols are known, the determination of QTLs for silk antibiotic compounds and corn earworm antibiosis will allow detection of useful markers for marker-assisted breeding, as well as an increased understanding of the genetic and cellular mechanisms involved in quantitative trait expression.

Genetic mechanisms underlying quantitative trait expression

Byrne *et al.*,[31] Lee *et al.*,[38] and McMullen *et al*.[49] used flavone synthesis as a model for understanding the genetic mechanisms underlying quantitative trait expression. To achieve this goal, these authors used RFLP markers in several maize mapping populations derived from the crosses of inbreds that differed considerably in antibiotic flavonoid compound concentrations. McMullen *et al.*[49] pointed out that genetic control of quantitative variation for maysin involved the interactions of at least four factors: the level of transcription activation of the pathway by the transcription regulator *p1*, the variation in the activity of enzyme-encoding genes, the flow of shared intermediates between distinct but connected pathways, and the separation of shared enzyme steps into independently regulated complexes allowing independent synthesis of very similar products.

Grotewold *et al.*[32] demonstrated that *p1*, a transcription activator, is sufficient to induce the pathway that leads to the accumulation of C-glycosyl flavones. In the population (GT114 × GT119) F_2, Byrne *et al.*[31] found that the *p1* locus accounted for 58% of the total phenotypic variance for maysin, and was dosage-dependent. The same authors suggested that additivity at *p1* may result from enhanced transcription of *p1*-regulated genes. The results were in agreement with those obtained by Butron *et al*.[35] in the population (GT-A1 × GT119) F_2, where the p1 locus explained about 50% of the phenotypic variance for maysin and 3'-methoxymaysin plus apimaysin. In the same population, the *p1* locus accounted for 28.3% of the phenotypic variability for chlorogenic acid content as Grotewold *et al*.[32] demonstrated in ectopic expression of the transgene of *p1*. McMullen *et al.*[49] concluded that, in any population in which parents differ by having functional and nonfunctional *p1* alleles, a substantial fraction of the variation for maysin would be expected to map to the *p1* region.

There were studies suggesting that the importance of the *whp1* locus, which encodes chalcone synthase,[55] was the most obvious candidate gene to explain the effect of chromosome 2L on maysin content and larval weight in (GE37 × FF8) $F_{2:3}$ families.[38] In the same study, it was stated that *sm1* on chromosome 6L could be involved in maysin synthesis, a locus that apparently controls the addition of a rhamnose molecule to the C-glycosyl group.[49] The locus *pr1*, which encodes 3'-hydroxylase,[56,57] was reported by McMullen *et al*.[49] as a possible major QTL for apimaysin synthesis in the (GT114 × NC7A) F_2 population.

The effect of the *a1* locus on maysin content is a good example of the flow of shared intermediates between connecting pathways. Styles and Ceska[29] reported that the *A1* allele is required in the pathways of anthocyanins and phlobaphenes, but not in the pathway of *p1* regulated C-glycosyl flavones. The *a1* locus encodes dihydroquercetin reductase, an enzyme acting in the pathway leading to phlobaphenes and 3-deoxyanthocyanins.[58] Nevertheless, in a subsequent report, Styles and Ceska[30] showed that the *a1* locus apparently enhances accumulation of C-glycosyl flavones in pericarp, suggesting that there could be a flow of shared intermediates between distinct but connected pathways. A block at the *a1* step in the pathway leading to phlobaphenes and 3-deoxyanthocyanins presumably leads to a build-up of flavone and other intermediates, some of which then are shunted into other branches of the pathway.[59] Guo *et al*.[33] reached the same conclusion when they studied the QTL involved in the synthesis of maysin in the (SC102 × B31867) F_2 population as McMullen *et al*.[36] when they studied the (W23a1 × GT119) F_2 population, since in both mapping populations *a1* was detected as a major QTL for maysin. Another example to illustrate the interrelationship between connecting pathways was supplied by Byrne *et al*.[31] These authors proposed that the QTL for maysin content found on chromosome 9S in the (GT114 × GT119) F_2 population could correspond to the *bp1* (brown pericarp1) gene, having probable activity in the pathway leading to 3-deoxyanthocyanins and phlobaphenes.

McMullen *et al.*[49] and Lee *et al*.[38] proposed that, in the flavonoid pathway, there is a separation of shared enzyme steps into independently regulated complexes allowing independent synthesis of very similar products. They detected a QTL for apimaysin synthesis and a different QTL for maysin synthesis in the (GT114 × NC7A) F_2 population, indicating that the syntheses of these compounds were independently based on the activities of these enzyme-encoding genes. Byrne *et al*.[60] hypothesized that closely related compounds such as maysin and apimaysin occur independently. However, epistatic interaction between both QTLs was significant for total flavones, suggesting the existence of a ceiling regulating the total possible amount of C-glycosyl flavones.[38] Butron *et al*.[35] reported the same QTLs for maysin and 3'-methoxymaysin plus apimaysin in the (GT-A2 × GT119) F_2 popula-

tion, but did not find any evidence to support the existence of competition among C-glycosyl flavones for limited substrates. QTL analysis results agreed with those obtained previously. Widstrom *et al*.[39] reported that maysin and two of its analogues (3'-methoxymaysin and apimaysin) are inherited similarly, and positive correlation coefficients between them suggests that they may exist in a chemical equilibrium rather than as one compound accumulating at the expense of the other.

Genetic action of quantitative trait loci for silk antibiotic compounds

A unique QTL was found on chromosome 5L for apimaysin content in the (GE37 × FF8) F_2 population.[38] Three RFLP markers for 3'-methoxymaysin plus apimaysin were identified on chromosomes 1S, 2L, and 8L in the (GT-A1 × GT119) F_2 population.[35] In the same study, the same QTLs plus one on chromosome 3S were detected for the synthesis of chlorogenic acid. Some of the chromosomal regions detected for the synthesis of maysin and other silk antibiotic compounds could correspond to the chromosomal regions identified for corn earworm resistance in previous studies. Robertson and Walter,[61] using the gene-marker translocation technique, identified genes for resistance to corn earworm on chromosomes 1, 3, 5, and 10 in the sweetcorn inbred line 245. Widstrom and Wiseman[62] located genes that contribute substantially to resistance on chromosomes 4 and 5 of inbred 245, on chromosome 3 of inbred 20, and on chromosome 8 of inbred La2W. Chromosomes 4 and 8 for inbred 20, 1 and 3 for inbred 81-1, and 6 for inbred 322 were implicated less strongly in conferring resistance to corn earworm.

The additivity of *p1* alleles for maysin, apimaysin, and 3'-methoxymaysin concentrations has been reported by several authors.[24,31,35,49,59] Two other major QTLs for maysin (*rem1* and *a1*) and a major QTL for apimaysin (*pr1*) had a recessive mode of action for high antibiotic compound concentrations[34,36,38] and epistatic interactions were significant in most of the crosses studied. These results confirmed those obtained from conventional inheritance studies. Widstrom and colleagues[39,63] found that more than one locus affects silk maysin concentration and additive effects are, in general, more important than non-additive effects. In a previous study, Widstrom *et al.*[64] found that non-additive genetic effects could be about equal to additive effects, but in their study maysin was measured spectrophotometrically and values were biased on the interference of other flavonoids.[65]

In the (GT114 × GT119) F_2 population, silk maysin concentration and corn earworm antibiosis were under similar genetic control and the *p1* locus played the major role in determining variation for both traits.[31] Chromosomal regions 1S, 2L, 6S, and 6L were also involved at the same time in the synthesis of maysin and antibiosis against corn earworm larvae.[31,66] However, some major QTLs reported for maysin were not always detected as QTLs for antibiosis against corn earworm larvae, and a lack of correlation between the increased flavone levels through *rem1* and pr1 and larval weight suppression was observed in the (GT114 × NC7A) F_2 population.[38] These data suggested that either the additional flavones were made at the expense of other antibiotic compounds, or that the population baseline flavone level was sufficient to cause larval death.

Development of high silk maysin *sh2* sweet corn

The long-term objective is to identify and examine the QTL associated with silk maysin synthesis and utilize these QTLs as markers to transfer maysin genes to the commercial sweet corn elite lines. The flavor of *sh2* super-sweet corn is preferred by consumers. Twenty-two years after Laughnan[13] predicted that *sh2* may be useful, over 90% of Florida sweet corn was super-sweet.[67] Unfortunately, *sh2* sweet corn has very little genetic variation for resistance to insects. To study insect resistance in sweet corn germplasm, Robertson and Walter[61] initiated a translocation linkage study in an attempt to locate genes that might be responsible for the resistance to the corn earworm in sweet corn lines. Widstrom and Wiseman[62] conducted a similar study involving six sweet corn inbreds. Both studies located a gene on chromosome 5 and Widstrom and Wiseman[62] detected another gene on the long arm of chromosome 4 for resistance to this insect. Guo *et al*.[34] demonstrated that the *P1* allele can have a major role in the resistance of *sh2* sweet corn to corn earworm, an allele that was lost in the historical development of sweet corn because of its pleiotropic effect on the undesirable cob color and silk browning.

In the (GE37 × 565) F_2 population,[34] the *P1* allele from the donor parent accounted for 61% of the phenotypic variation of maysin concentration, and the means of maysin for different genotypic classes showed that *P1* acted in additive fashion on maysin concentration. The *a1* locus from the recurrent parent is the second important QTL detected in this population, accounting for 6.4% of the phenotypic variation, which acted in a recessive manner for high maysin.[33,36] The epistatic interaction between *p1* and *a1* on maysin and AM-maysin concentrations was demonstrated clearly in this study and others, in which recombined individuals with *P1* from one parental line and *a1* from another parental line have two to three times higher silk flavones (maysin and AM-maysin) than the donor parental line GE37. These results are consistent with the findings in the genetic strains and stocks of *p1* and *a1* reported by Styles and Ceska,[29,30] in the (GT114 × GT119) F_2 population by Byrne *et al.*,[31] and in the *p1*-transgenic BMS suspension cells by Grotewold *et al*.[32] In a similar study, Guo *et al.*[33] reported the QTLs associated with silk maysin in the F_2 population of (SC102 × B31857) (field corn × *sh2* sweet corn). They detected two markers (*npi286* and *csu3*) flanking to the *p1* locus on chromosome 1. The marker *npi286* explained 25.6% of silk maysin variance and *csu3* explained 17.9% of the phenotypic variance in the region of *p1* locus.

Guo *et al*.[34] also delineated the roles of *p1* and *a1* as having quantitatively genetic control over chlorogenic acid.[68] Specifically, the results support the suggestion by Grotewold *et al*.[32] that the *p1* gene regulates the synthesis of chlorogenic acid. Antibiotic activity of chlorogenic acid against corn earworm is equivalent to that of maysin because of the structural similarity.[26,27] Apimaysin and 3'-methoxymaysin have antibiotic activities equivalent to about 50% of that for maysin. Therefore, improvement of chlorogenic acid and AM-maysin (apimaysin and 3'-methoxymaysin) may be important to the capacity of host plants' resistance to insects when individuals have a substantial amount of these minor compounds.[19]

The effect of the *a1* gene shows dominant gene action for low maysin and significant epistatic gene action with the *p1* gene.[19] The increases in concentrations of maysin, AM-maysin, and chlorogenic acid may be explained. 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.[52] The *A1* encodes for a dihydroflavonol 4-reductase.[58,69] This enzyme is involved in two branches of the flavonoid pathway (Figure 1) and converts dihydroflavonols as well as flavanones.[29,30] Dihydroflavonols are the precursors of 3-hydroxyanthocyanins, whereas flavanones are precursors of the 3-deoxyanthocyanins and phlobaphenes. Styles and Ceska[29] reported that the *A1* allele is required in the pathways leading to anthocyanins and 3-deoxyanthocyanins, but not in the pathway of *p1* regulated flavones. 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.[29,30] Therefore, a block at the a1 locus could accumulate the intermediate flavanones, which are available for biosyntheses of C-glycosyl flavone (e.g. maysin) and chlorogenic acid, as demonstrated by a recent study[59] using *Arabidopsis* as a model for studying the flavonoid biosynthet-

ic pathway regulation. Pelletier *et al*.[59] revealed that mutant lines blocked at intermediate steps of the pathway actually accumulated higher levels of specific flavonoid enzymes and other end products.

The presence of maysin and its analogues with antibiotic activity in silks is an important defense against invasion of the ear by corn earworm in the Southern United States. A thorough knowledge of the inheritance of these compounds will assist breeders in choosing the most efficient method of incorporating this trait into elite sweet corn inbred lines. Silk maysin concentrations above 0.2% begin to substantially reduce larval growth and prevent completion of the life cycle when husk coverage is sufficient to force the insect to feed on silks while entering the ear.[41] Chlorogenic acid and two maysin analogues, apimaysin and 3'-methoxymaysin, were found in such minor quantities in this mapping population that they could not be credited with any substantial impact on antibiotic activity against the corn earworm as tested by Guo *et al*.[19] Because of the close linkage of *a1* and *sh2*[52,70] and the association of high maysin with the homozygous *sh2* trait,[19] the plant breeder could introgress resistance to the corn earworm into elite *sh2* sweet corn material,[71] in which Scully *et al.*[71] selected and released a high maysin *sh2* sweet corn germplasm. If silk browning and cob color are critical factors for maysin production in sweet corn but lack the customer's preference, then separating the red cob and browning silk, which are controlled by the *P1* allele, may be difficult if not impossible. The high maysin lines selected from the cross of (GE37 × 565) are clear pericarp, red cob, and browning silk. There is some field corn germplasm with *p1-wwr* alleles (clear pericarp, white cob, browning silks) (personal communication with NW Widstrom), but the amount of antibiotic flavones and their potential as a donor need further investigation.[39]

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