PHENOTYPIC AND GENOTYPIC EVALUATION OF GENERATIONS AND RECOMBINANT INBRED LINES FOR RESPONSE TO AFLATOXIN

A Dissertation

by

HALIMA THELMA BELLO

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2007

Major Subject: Plant Breeding

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ABSTRACT

Phenotypic and Genotypic Evaluation of Generations and Recombinant Inbred Lines for Response to Aflatoxin. (May 2007)

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Aspergillus flavus is a fungus pathogen of maize that causes contamination of maize with aflatoxin. Aflatoxin is a carcinogenic toxin that can cause harm to human and animal health. Several management practices have been developed, such as cultural, chemical, biological and breeding, for host resistance. Development of host plant resistance has been the most desirable but this has been hampered by several factors, such as environmental influence, time consuming phenotyping and costly inoculation and field evaluations. Because of the problems associated with breeding for aflatoxin resistance, heritability estimates along with genetic correlations for aflatoxin and its secondary traits were estimated in this study. This experiment was conducted in two Texas locations (College Station and Weslaco) and phenotypic data were collected for aflatoxin concentration, maturity, endosperm texture, percentage of rotten ears and grain yield per ear.

The heritability was moderate to high for aflatoxin and secondary traits such as endosperm texture and percentage of rotten ears. Aflatoxin was observed to be negatively correlated to grain yield and positively correlated to percentage of rotten ears. A population of recombinant inbred line derived from a cross between CML161 and B73*o2* were evaluated in replicated trials in two environments for resistance to aflatoxin contamination. The families were genotyped using simple sequence repeat (SSR) markers. The mapping Recombinant Inbred Line population was used to detect and characterize Quantitative Trait Loci associated with aflatoxin accumulation. Alleles for

reducing aflatoxin contamination came from both parents across the chromosomes. Thirty-eight epistatic interactions were detected for aflatoxin resistance.

Several other QTLs were identified for other traits such as grain yield, endosperm texture, and percentage of rotten ears. The QTLs reported in this experiment are promising and need to be validated in other environment and genetic backgrounds for further use in marker assisted selection.

Inheritance studies using generation mean analysis in six crosses showed additive and dominance effects to be mainly responsible for aflatoxin resistance. Two inbreds, CML176 and CML161, were identified as sources of resistance in breeding programs and use for selection.

DEDICATION

This dissertation is dedicated to my mother Mrs Comfort Atta who despite the early death of my Dad supported me in all ramifications and to my husband Dr Steven Bello whose love and support kept me going.

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I would like to thank most sincerely Dr. Javier F. Betrán, my major advisor and chair of my graduate committee, for his guidance and support not only in accomplishing this research work and doctoral level studies here at Texas A&M University but also in other aspects throughout the entire stay here in the United States. I appreciate all the help he availed to me while pursuing my studies; he showed a good example of hard work. I would also like to express my gratitude to members of my graduate committee: Dr. William L. Rooney, Dr. Monica Menz, and Dr. Tom Isakeit for their guidance and constructive comments during the course of my studies.

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I would also like to acknowledge the understanding of my family during my studies and stay at Texas A&M. My husband Steven (Honey, you are great). Thanks also to my siblings Yakubu, Hussena, Hassan, and Inda. Hussena, you are more than a sister, you were a trustworthy and understanding friend.

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I wish to also express my gratitude to my very friend Ndmanbe, you have been very supportive. Other close acquaintances such as Toyin, Momsie, Rose and Leo are all appreciated.

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TABLE OF CONTENTS

Page

CHAPTER Page

LIST OF FIGURES

FIGURE Page

LIST OF TABLES

TABLE Page

TABLE Page

TABLE Page

TABLE Page

CHAPTER I

INTRODUCTION

Maize (*Zea mays* L.) is ranked third among the world cereal crop production after rice and wheat. It is the most important cereal crop in the United States, which is the largest producer of maize in the world. While most of the corn grown in the United States is produced in the Midwestern States while a significant quantity is produced in the Southern U.S. The United States maize production is composed of 95% of dent yellow type, which is mostly used for livestock. The remaining type of maize produced is constituted of sweet, high amylase, popcorn and colored maize, which are used for human consumption. Maize is known to contribute 15% of protein and 20% of calories derived from food crops in the world's diet (National Research Council, 1988). Maize is used as food, feeds, fuel, and starch products in different parts of the world. Maize is a staple food and an important source of protein in developing countries such as Africa, Asia and Latin America.

The quantity of protein in maize is poor due to deficiency in the essential amino acids lysine, tryptophan and methionine (Watson, 1988). The poor quality protein in maize is due to the high concentration of zeins storage proteins (50 – 60%) which are devoid of lysine and tryptophan. Wilson (1991) reported other storage proteins in maize endosperm such albumins (3%), globulins (3%) and glutelins (30 – 45%) consisting of higher lysine content of 5 – 6%, 5 – 8% and 4 – 5% than zeins, respectively.

Maize is frequently infected with fungi which produce toxins that affect the quality and safety of food and animal feeds. One of the serious sources of maize contamination is the fungi called *Aspergillus flavus* and this is responsible for severe economic losses in maize in the South and Midwestern United States.

This dissertation follows the style and format of Crop Science.

This fungus produces a potent carcinogen called aflatoxin, which is dangerous to human and animal health causing liver cancer (Castegnaro and McGregor, 1998).
Aflatoxin contamination has been associated with high temperature, drought stress and insect injury (Payne, 1992, 1998). Several management practices have been employed in the control of aflatoxin contamination, and they include cultural, chemical, physical, biological and breeding methods. However, no control strategy is completely effective when environmental conditions are extremely favorable for the growth of the fungus. The most desirable method of aflatoxin control is through host plant resistance.

Resistance to aflatoxin is affected by several factors such as the huge influence of environments, high cost of phenotyping and inoculation, lack of rapid screening method and resources for extension field evaluations. The first and most important step in breeding for resistance to complex traits such as aflatoxin accumulation is to identify sources of resistance that have agronomically valuable characteristics. Other strategies include identification of secondary correlated traits and the use of marker assisted selection. The information has led to the need to undertake this study. Therefore, the objectives were to:

- To investigate the genetic basis of resistance of selected inbreds and to identify which of the inbreds are the most promising sources of aflatoxin resistance?
- To estimate heritability and genetic correlations between aflatoxin accumulation and secondary traits in a recombinant inbred line population.
- To identify QTL's for the phenotypic variation of aflatoxin accumulation and its secondary traits.

CHAPTER II
GENERATION MEAN ANALYSIS OF AFLATOXIN ACCUMULATION

Introduction

Aflatoxin is a potent toxin and carcinogen produced by the fungus *Aspergillus flavus* that can cause aflatoxicosis and liver cancer in animals and humans (Castegnaro and Mc Gregor, 1998). Pre-harvest aflatoxin contamination during flowering and grain filling period is a major problem for maize growers in Texas and other areas in the U.S. and the world.

Generation mean analysis (GMA) is a genetic design used to characterize the inheritance of a relevant trait and by identifying the types of gene action conditioning the target trait when crossing two parental inbreds commonly having contrasting expression of the trait. Studies on the inheritance of resistance are necessary to determine the gene action controlling resistance so that appropriate breeding procedures can be developed. In disease resistance studies, before the crosses are made to generate the different generations, it is important to identify susceptible and resistant parents. This is especially important in GMA as it is based on the assumption that the parental values will be significantly different and the genes for resistance are located in the resistant parent. Compared to other mating designs GMA has several advantages such as its small sized experiments allows certain levels of precision and errors are reduced when working with means rather than with variances.

Generation means analysis has been used to study quantitatively inherited traits in corn including disease resistance (Carson et al., 1981; and Darrah et al., 1987). Inheritance of resistance to aflatoxin production in corn has been reported in several studies (Campbell et al., 1997; Campbell et al., 1993; and Gorman et al., 1992). Identification of sources of resistance and determining the gene action is importance in studying the genetics of aflatoxin resistance.

In this study, Quality Protein Maize (QPM) materials. Were used as breeding materials. QPM is an *opaque2* high lysine and tryptophan maize with hard vitreous endosperm. That was developed over many years of selection for reduced ear rot and lysine content. Information on the level of resistance and mode of inheritance on QPM inbreds will facilitate introgression from these sources into potential elite germplasm and fasten the development of appropriate breeding procedures. Therefore, the objectives of this study were

- To investigate the genetic basis of the degree of resistance of selected inbreds through generation means analysis, and
- to identify which of the inbreds are the most promising sources of resistance for conventional and molecular marker assisted breeding.

Review of Literature

Quality Protein Maize

The protein in maize is nutritionally poor with a biological value of 40 – 57% because it is deficient in amino acids lysine and tryptophan, which are essential for monogastric animal and humans is (Bressani, 1992). High lysine maize has greater lysine and tryptophan content (60 – 100%) in maize kernels and a higher biological value of about 80%, its endosperm has the *opaque-2* gene which regulates transcription of alpha zeins class of kernel storage proteins reducing 22kd and 19kd fractions with a resulting increase in other lysine rich non – zein fractions by 40% (Moro et al., 1996). Unfortunately, *opague-2* maize has poor agronomic and kernel characteristics such as low grain yield, high root and stalk lodging along with greater susceptibility to ear roots and stored grain pests one of which is aflatoxin (Vasal, 2001). This led to the development of Quality Protein Maize (QPM) by the International Center for Maize and Wheat Improvement (CIMMYT). QPM was developed by introducing modifier genes and selecting for a hard vitreous endosperm in *o2/o2* germplasm (Vasal, 2001). Compared to normal maize QPM has a much superior protein quality (82.1 % as percent casein).and it is postulated that the food processing and animal industries of the state of

Texas alone could benefit by almost $80 million per year by replacing normal maize with QPM (TAES, 1990).

Several QPM germplasm are more adapted to tropical and subtropical environments and less adapted to temperate regions.

Inheritance Studies

A number of sources of relative resistance to *Aspergillus* ear and kernel rot and inhibition of aflatoxin production have been identified by screening and selection (Campbell and White, 1994, 1995a, 1995b; Scott and Zummo 1988, 1990, 1992) Resistance to *Aspergillus* ear rot and aflatoxin production from previously studied sources of resistance is quantitatively regulated. Most of these studies have focused on the evaluation of inbreds and their hybrids using diallel mating designs. General combining ability is accepted as being more important for resistance to aflatoxin production than specific combining ability, which suggests additive gene action controlling resistance (Darrah et al., 1987; Gorman et al., 1987; Widstrom et al., 1987; Zuber et al., 1978). Based on what is known about the genetic basis for resistance to *A. flavus* and aflatoxin production from previous studies, it is advisable to closely examine genetic relationships and interactions among the more promising new resistance sources.

Large genotype x environment interactions of aflatoxin accumulation has been reported in various studies (Hamblin and White, 2000 and Widstrom and McMillian, 1984). Generation means analysis for some sources of resistance showed that additive effects were important, whereas dominance effects were significant (Campbell, 1995b). These observations indicate the need and merit of evaluating potential and promising sources of resistance to aflatoxin accumulation in different genetic studies and in different environment before general, reliable conclusions are made on a particular source of resistance.

Material and Methods

Germplasm and Environments

A total of six inbreds were used to generate six crosses for generation means analysis and procedure and each cross consists of six generations namely P_1, P_2, F_1, F_2, BCP_1 and BCP_2. The F_1, F_2, BCP_1 and BCP_2 generations were produced by crossing the parental inbred lines, selfing individual F_1 plants, crossing the F_1 to the susceptible parent and crossing the F_1 to the resistant parent. Some of these generations are genetically homogenous (P_1, P_2, F_1) and the rest are segregating (F_2, BCP_1, BCP_2). The six inbreds were QPM lines CML161 and CML176, and tropical white line CML269, all three released by CIMMYT, and Texas inbreds Tx804, Tx114 and Tx811, which are Southern USA lines and were released or developed by the Texas Agriculture Experiment Station. The generations were developed from six biparental crosses: CML176 x Tx811, CML161 x Tx811, CML161 x Tx804, CML176 x Tx114, CML269 x Tx114, CML78 x CML176, and CML269 x CML176. Each cross was comprised of two parental lines with contrasting response to aflatoxin accumulation. The six generations (P_1, P_2, F_1, F_2, BCP_1, and BCP_2) for the six crosses were evaluated in 2003 with replicated trials at two locations in Texas, Weslaco (latitude 26^o09, elevation 82.5m) and College Station (latitude 30^o37, elevation 96m). Twice the numbers of observations were recorded in the segregating generations as compared with the non-segregating generations. One row plot for the non segregating population and two row plot for the segregating population. In each row three plant samples were bulked. The experimental design used was randomized complete design with two replications in each environment.

Maize Inoculation and Aflatoxin Quantification

The traits were inoculated with *Aspergillus flavus* isolate NRRL3357. The method of inoculation used was the non-wounding silk channel inoculation technique (Zummo and Scott, 1989). The plants were inoculated six to ten days after midsilk using a conidial suspension containing 3×10^7 conidia of *A. flavus* in 3ml of distilled water injected into the plant. One ear per plot was ground using a Romer mill and 50 g sub samples were

used to quantify aflatoxin using monoclonal antibody affinity columns for purification and fluorescence determination for analysis following the Vicam Aflatest protocol (Watertown, MA). Aflatoxin concentration were reported in parts per billion (ppb) or (ng g^{-1}).

Statistical Analysis

Aflatoxin concentration (ppb) was transformed using logarithm to equalize variance. This transformation is commonly used with aflatoxin concentrations. Both logarithmic and untransformed aflatoxin values were used in the analysis and presentation of results. Adjusted means were analyzed using $REML_{tool}$ software. The following genetic effects were estimated using SAS procedures: a = additive effects, d = dominance effects, ad = additive by dominant effects, and aa = additive by additive, and dd = dominant by dominant epistatic effects, following Gamble (1962).

Results

Generation Mean Analysis for the Tx811 by CML176 Cross

Significant differences were observed among generations. Aflatoxin production was higher in College Station than in Weslaco (Table 2.1). The F1 generation means were significantly higher than the other generations in both locations (Figure 2.1). Inbred Tx811 had higher concentrations of aflatoxin when compared to CML176. These results are similar to previous evaluations where CML176 had less susceptibility to aflatoxin (Betran et al., 2005). Inbred CML176 and its backcross generation showed reduced levels of aflatoxin, suggesting that it has resistant factors that are heritable. In this cross, a large portion of the genetic effects seems to be due to epistasis (Table 2.2). Dominance by dominance epistatic interaction was the most prevalent of all the genetic effects.

Table 2.1. Means of various generations derived from the cross between inbreds Tx811 and CML176 at College Station and Weslaco, TX in 2003.

Pedigree	Weslaco		College Station	
	Aflatoxin ppb	logAF log ppb	Aflatoxin ppb	logAF log ppb
(Tx811 x CML176)	277.90	2.18	327.16	2.29
(Tx811 x CML176)/Tx811	38.00	1.51	95.16	1.90
(Tx811 x CML176)/CML176	45.10	1.56	220.33	2.14
Tx811 x CML176	104.00	1.99	730.00	2.81
Tx811	166.70	2.16	216.33	2.13
CML176	81.00	0.96	87.66	1.70
MEAN	118.78	1.72	279.44	2.16
STDEV	90.79	0.47	238.11	0.37

Table 2.2. Least square estimates of genetic parameters used in the generation mean analysis of the cross between Tx811 and CML176 evaluated for aflatoxin accumulation at College Station and Weslaco, TX in 2003

Trait	Parameter	Parameter Estimates		
		College Station	Weslaco	Across
Aflatoxin (ng g^{-1})	m	327.16**	277.81**	302.53**
	a	125.16	7.10	66.13
	d	-99.66	-965.17*	-532.42
	aa	-677.66	-945.33*	-811.50
	ad	189.50	49.94	119.72
	dd	1810.66*	1234.78*	1522.72**
Aflatoxin (log ng g^{-1})	m	2.29**	2.16**	2.23**
	a	0.24	0.05	0.15
	d	-0.21	-2.05	-1.13
	aa	-1.11	-2.49	-1.80*
	ad	0.46	0.65	0.56*
	dd	2.46	3.45	2.96*

**** Significant at 0.01 and * at 0.05**

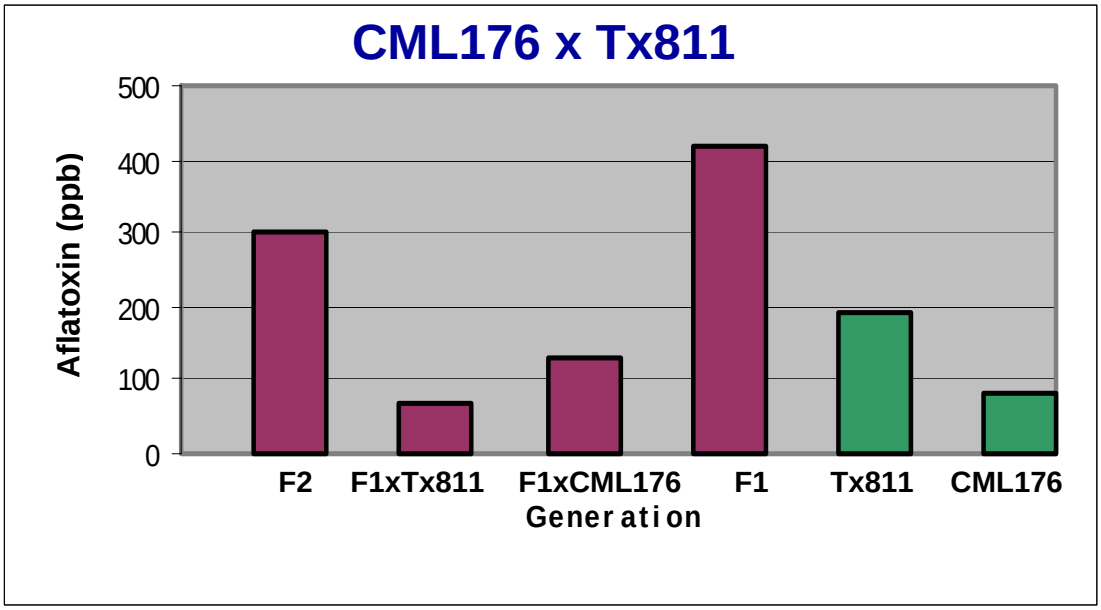

Figure 2.1. Means across locations (College Station and Weslaco, TX) for different generations of the cross between Tx811 and CML176 in 2003.

Generation Mean Analysis for the Tx804 by CML 161 Cross

In the cross between Tx804 by CML 161, aflatoxin production was greatest in College Station as compared to Weslaco (Table 2.3). The means of aflatoxin for the different generations (Fig 2.2) showed that in both locations inbred Tx804 means were significantly higher than all other generations confirming reports from previous evaluations that inbred Tx804 is susceptible to aflatoxin. The other parent CML161 and backcrosses with CML161 as recurrent parent showed reduced levels of aflatoxin, suggesting that it has heritable resistant factors. Only additive by dominance epistatic interaction was significant in College Station (Table 2.4). In all other locations, least squares estimates of genetic effect were not significant in Weslaco and across locations.

Table 2.3. Means of various generations derived from the cross between inbreds Tx804 and CML161 at College Station and Weslaco, TX in 2003.

	Weslaco		College Station	
Pedigree	Aflatoxin ppb	logAF log ppb	Aflatoxin ppb	logAF log ppb
(Tx804 x CML161)	149.40	2.10	896.66	2.70
(Tx804 x CML161)/ Tx804	254.90	2.29	921.66	2.82
(Tx804 x CML161)/CML161	150.30	1.97	410.00	2.49
Tx804 x CML161	40.1	1.52	206.66	2.25
Tx804	646.7	2.66	3900.00	3.58
CML161	40.0	1.32	640	2.67
MEAN	213.56	1.97	1162.50	2.75
STDEV	227.00	0.49	1369.34	0.45

Table 2.4. Least square estimates of genetic parameters used in the generation mean analysis of the cross between Tx811 and CML 176 evaluated for aflatoxin accumulation at College Station and Weslaco, TX in 2003.

Trait	Parameter	Parameter Estimates		
		College Station	Weslaco	Across
Aflatoxin (ng g^{-1})	m	896.67**	149.44*	523.06*
	a	-511.67	-104.66	-308.16
	d	-2986.67	-90.55	-1538.61
	aa	-923.33	212.67	-355.33
	ad	1118.33	198.67	658.50
	dd	3213.33	-256.22	1478.56
Aflatoxin (log ng g^{-1})	m	2.70**	2.10**	2.40**
	a	-0.32	-3.32	-0.32
	d	-1.05	-3.34	-0.70
	aa	-0.18	0.12	-0.03
	ad	0.13	0.34	0.24
	dd	0.32	-1.62	-0.65

**** Significant at 0.01 and * at 0.05**

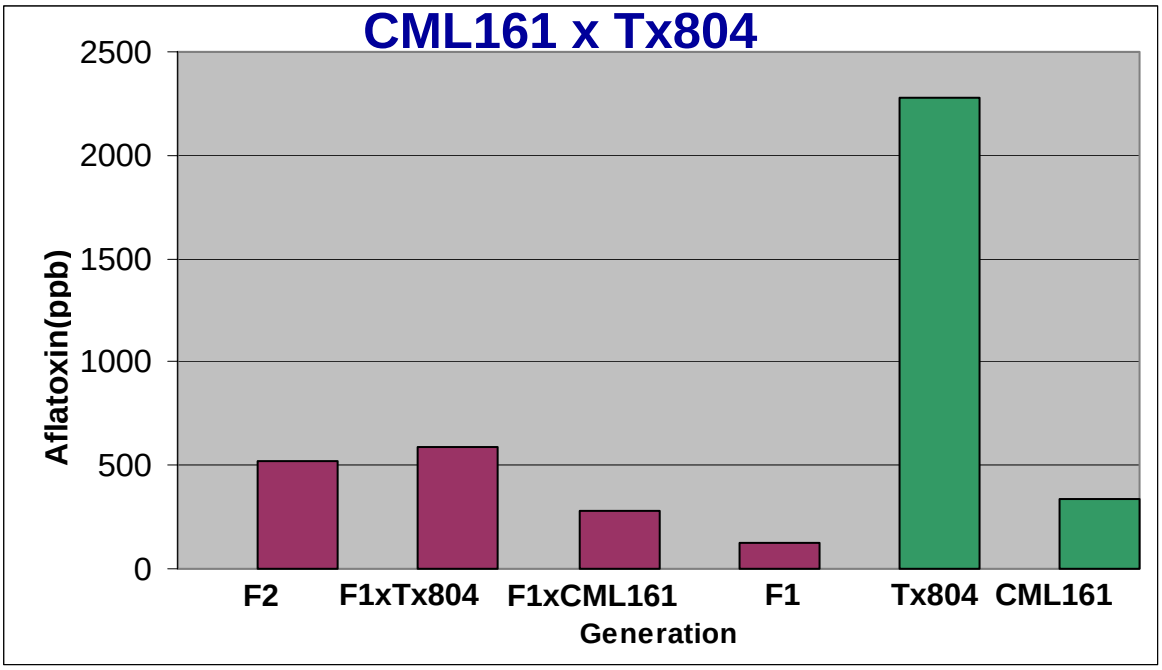

Figure 2.2. Means across locations (College Station and Weslaco, TX) for different generations of the cross between Tx804 and CML161 in 2003.

Generation Mean Analysis for the Tx114 by CML 176 Cross

As in the previous crosses, aflatoxin accumulation was greatest in College Station than Weslaco. The means showed that Tx114 had significantly higher mean than the other generations in both locations (Fig 2.3). The distributions were skewed towards the resistant parent CML 176 whose generations showed reduced levels of aflatoxin. The backcross to the susceptible parent Tx114 had the highest mean of 553.3 ppb in College Station (Table 2.5). Least square estimates showed that additive and dominance effect were significant in College Station and across locations while in Weslaco only additive gene action was significant (Table 2.6). Additive and dominance gene actions seems to be of primary importance in this cross.

Table 2. 5. Means of various generations derived from the cross between inbreds Tx114 and CML176 at College Station and Weslaco, TX in 2003.

Pedigree	Weslaco		College Station	
	Aflatoxin ppb	logAF log ppb	Aflatoxin ppb	logAF log ppb
(Tx114 x CML176)	64.20	1.73	362.16	2.47
(Tx114 x CML176)/CML176	29.70	1.13	123.33	1.96
(Tx114 x CML176)/Tx114	100.70	1.74	403.33	2.48
Tx114 x CML176	28.60	1.20	47.00	1.63
CML176	7.70	0.68	308.66	2.04
Tx114	303.30	2.43	890.00	2.94
MEAN	89.03	1.48	355.74	2.25
STDEV	109.95	0.61	296.41	0.46

Table 2.6. Least square estimates of genetic parameters used in the generation mean analysis of the cross between Tx811 and CML176 evaluated for aflatoxin accumulation at College Station and Weslaco, TX in 2003.

Trait	Parameter	Parameter Estimates		
		College Station	Weslaco	Across
Aflatoxin (ng g^{-1})	m	362.17**	64.22*	213.19**
	a	280.00*	70.94*	175.47*
	d	947.67*	-123.06	-535.36
	aa	-395.33	3.88	-195.72
	ad	10.66	-76.88	-43.77
	dd	634.67	103.45	369.06
Aflatoxin (log ng g^{-1})	m	2.47**	1.73**	2.10**
	a	0.52*	0.61*	0.56*
	d	1.86*	-1.51	1.69*
	aa	-1.01	-1.15	-1.08
	ad	0.07	0.26	-0.09
	dd	0.37	0.92	-0.09

**** Significant at 0.01 and * at 0.05**

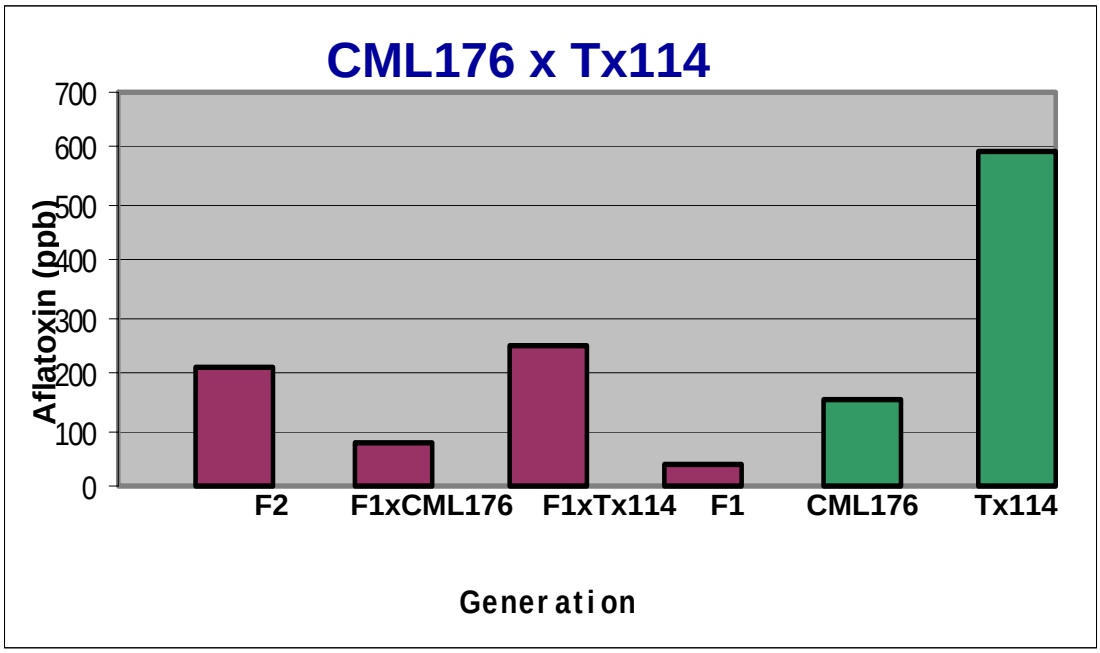

Figure 2.3 Means across locations (College Station and Weslaco, TX) for different generations of the cross between Tx114 and CML176 in 2003.

Generation Mean Analysis for the Tx114 by CML 269 Cross

As in the previous crosses, aflatoxin accumulation was greatest in College Station . The generation means showed that Tx114 had significantly higher mean than the other generations across both locations (Fig 2.4). Inbred CML269 and its backcross generations had lower concentration of aflatoxin when compared to Tx114 suggesting that it has resistant factors that are heritable (Table 2.7). The analysis of the generation means showed only additive gene action to be significant for aflatoxin (ppb) in College Station and across locations (Table 2.8).

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Table 2.7. Means of various generations derived from the cross between inbreds CML269 and Tx114 at College Station and Weslaco, TX in 2003.

	Weslaco		College Station	
Pedigree	Aflatoxin ppb	logAF log ppb	Aflatoxin ppb	logAF log ppb
(CML269 x Tx114)	417.70	2.52	182.83	2.17
(CML269 x Tx114)/CML269	293.10	2.07	210.66	2.22
(CML269 x Tx114)/Tx114	355.60	2.32	683.33	2.73
CML269 x Tx114	230.70	2.28	48.66	1.54
CML269	8.00	0.72	700.00	2.74
Tx114	425.00	2.50	1270.00	3.07
MEAN	288.35	2.06	515.91	2.41
STDEV	156.12	0.68	458.91	0.54

Table 2.8. Least square estimates of genetic parameters used in the generation mean analysis of the cross between CML269 and Tx114 evaluated for aflatoxin accumulation at College Station and Weslaco, TX in 2003.

Trait	Parameter	Parameter Estimates		
		College Station	Weslaco	Across
Aflatoxin (ng g^{-1})	m	182.83	417.72**	300.28**
	a	472.67*	62.49	267.58*
	d	120.33	-359.50	-119.58
	aa	1056.67	-373.67	341.50
	ad	187.67	-146.00	20.83
	dd	-777.33	-29.22	-403.28
Aflatoxin (log ng g^{-1})	m	2.17**	2.52**	2.34**
	a	0.50*	0.25	0.38
	d	-0.14	-0.62	-0.38
	aa	1.21*	-1.29	-0.04
	ad	0.34	0.64*	-0.14
	dd	2.22*	0.28	-0.97

**** Significant at 0.01 and * at 0.05**

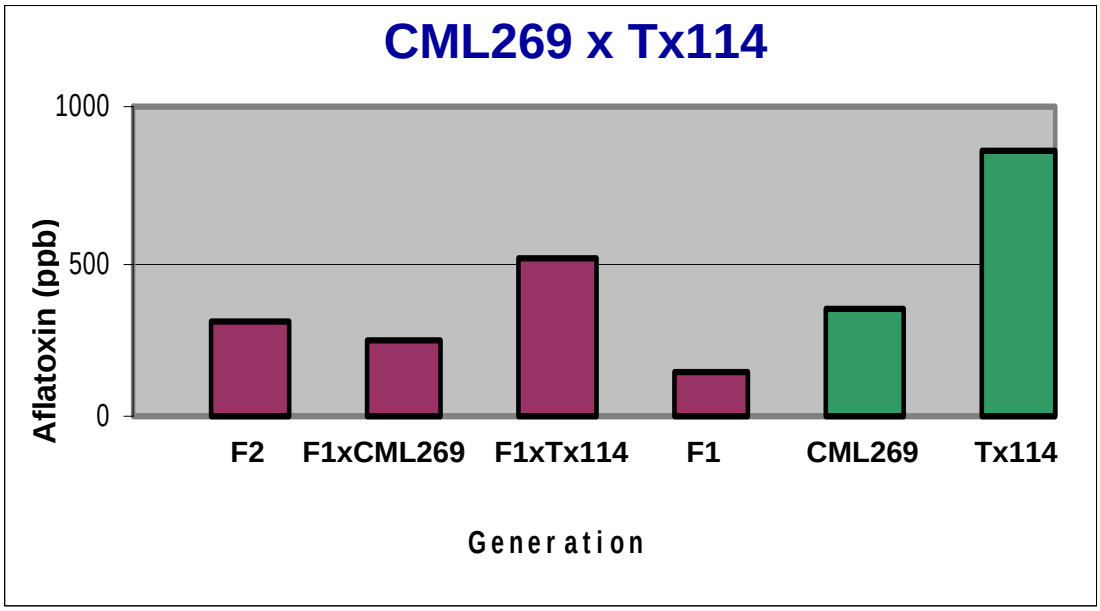

Figure 2.4. Means across locations (College Station and Weslaco, TX) for different generations of the cross between Tx114 and CML269 in 2003.

Generation Mean Analysis for the CML78 by CML176 Cross

In the cross between CML176 by CML78, aflatoxin production was highest in College Station (Table 2.9). The generation means distribution showed that inbred CML78 means were significantly higher across both locations (Fig 2.5). Inbred CML78 also had higher concentration of aflatoxin in both College Station and Weslaco. Inbred CML176 and its generations (F1s and BC1s) showed reduced levels of aflatoxin, suggesting that it has resistant factors that are heritable. Least square estimation of gene effects showed significance for dominance by dominance gene action to be significant in College Station, Weslaco and across both locations (Table 2.10). Across both locations additive by additive, additive by dominance and dominance by dominance gene actions were significant.

Table 2.9. Means of various generations derived from the cross between inbreds CML78 and CML176 at College Station and Weslaco, TX in 2003.

	Weslaco		College Station	
Pedigree	Aflatoxin ppb	logAF log ppb	Aflatoxin ppb	logAF log ppb
(CML78 x CML176)	48.50	1.48	116.33	2.02
(CML78 x CML176) / CML176	127.30	1.95	204.33	2.28
(CML78 x CML176) / CML78	95.40	1.79	311.00	2.27
CML78 x CML176	14.40	1.00	84.66	1.79
CML78	143.00	2.02	486.66	2.67
CML176	23.33	1.02	83.33	1.65
MEAN	75.32	1.54	214.38	2.11
STDEV	54.44	0.45	159.49	0.37

Table 2.10. Least square estimates of genetic parameters used in the generation mean analysis of the cross between CML78 and CML176 evaluated for aflatoxin accumulation at College Station and Weslaco, TX in 2003

Trait	Parameter	Parameter Estimates		
		College Station	Weslaco	Across
Aflatoxin (ng g^{-1})	m	116.23	48.49	82.41*
	a	-106.7	-61.83	-22.41
	d	365.1	242.73	303.86
	aa	565.42	311.45	438.39*
	ad	-308.4	-2.00	153.17*
	dd	-856.6**	621.66**	739.16*
Aflatoxin (log ng g^{-1})	m	2.02**	1.48**	1.75**
	a	0.00	-0.16	-0.08
	d	0.65	1.05	0.86
	aa	1.02	1.58	1.29*
	ad	0.50*	0.33	0.42
	dd	-2.20*	-4.03*	-3.11**

**** Significant at 0.01 and * at 0.05**

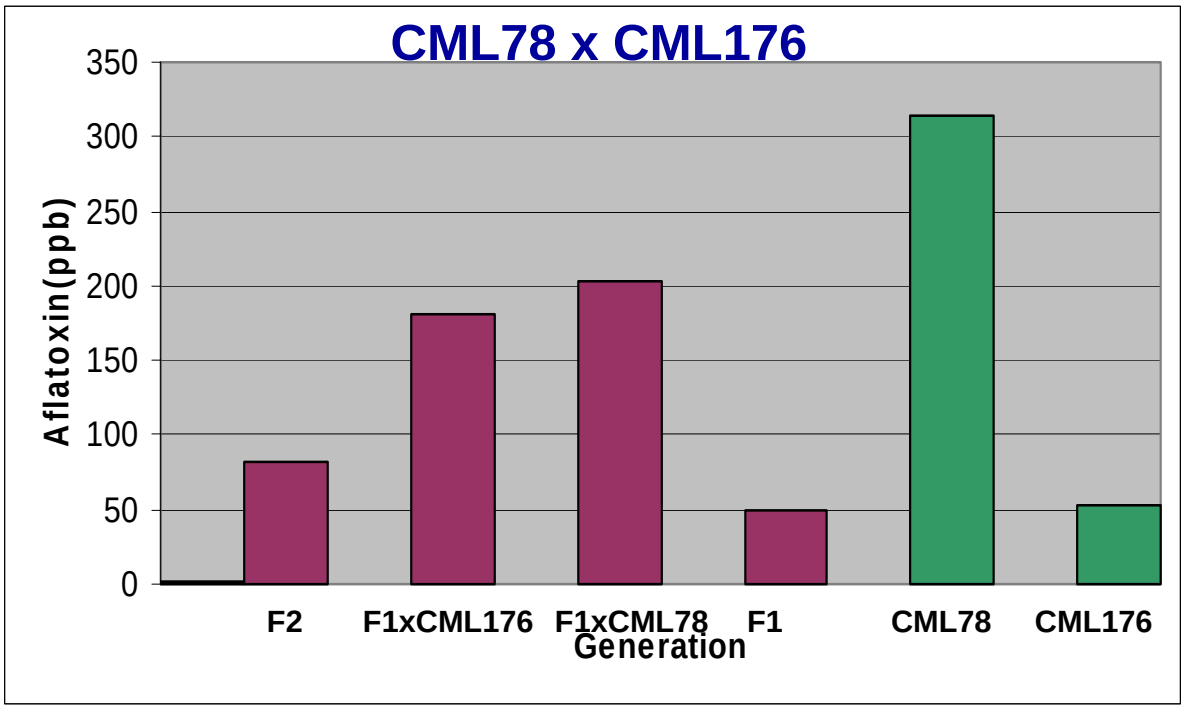

Figure 2.5. Means across locations (College Station and Weslaco, TX) for different generations of the cross between CML78 and CML176 in 2003.

Generation Mean Analysis for the CML269 by CML176 Cross

In this cross both lines are considered to be resistant to aflatoxin. These lines exchanged resistance positions among themselves at the two testing locations (Table 2.11). In Weslaco, CML 176 and its backcross exhibited high levels of aflatoxin concentration but in College Station and across locations CML 269 showed significantly higher level of aflatoxin concentration than CML 176. When performance of these two inbreds was compared across environments, CML 176 still had much lower level of aflatoxin accumulation (Fig. 2.6). In the analysis of the generation means additive gene action was significant for aflatoxin (ng g^{-1}) and logarithmic transformation of aflatoxin in College Station, Weslaco and across all locations. Dominance and additive by additive gene action was significant in Weslaco (Table 2.12).

Table 2.11. Means of various generations derived from the cross between inbreds CML269 and CML176 at College Station and Weslaco, TX in 2003.

	Weslaco		College Station	
Pedigree	Aflatoxin ppb	logAF log ppb	Aflatoxin ppb	logAF log ppb
(CML269 x CML176)	174.60	2.05	562.83	2.55
(CML269 x CML176)/ CML269	49.90	1.65	1177.07	2.82
(CML269 x CML176)/ CML176	27.40	1.16	456.24	2.39
CML269 x CML176	29.60	1.42	59.33	1.66
CML269	19.30	1.17	986.66	2.81
CML176	34.00	1.52	83.66	1.86
MEAN	55.8	1.49	554.29	2.34
STDEV	59.07	0.33	458.46	0.48

Table 2.12. Least square estimates of genetic parameters used in the generation mean analysis of the cross between CML269 and CML176 evaluated for aflatoxin accumulation at College Station and Weslaco, TX in 2003

Trait	Parameter	Parameter Estimates		
		College Station	Weslaco	Across
Aflatoxin (ng g^{-1})	m	562.83*	174.56**	368.70*
	a	-885.83*	-22.44	-399.59*
	d	501.17	-540.67**	-128.85
	aa	977.00	-543.56**	107.62
	ad	-434.33	-29.78	-177.51
	dd	-3016.33	501.34*	-1039.29
Aflatoxin (log ng g^{-1})	m	2.55*	2.05**	2.30**
	a	-0.65*	-4.48*	-0.51*
	d	-0.24	-2.51*	-1.49
	aa	0.43	-2.61**	-1.21
	ad	-0.17	-0.64*	-0.34
	dd	-3.05*	2.49*	-0.04

**** Significant at 0.01 and * at 0.05**

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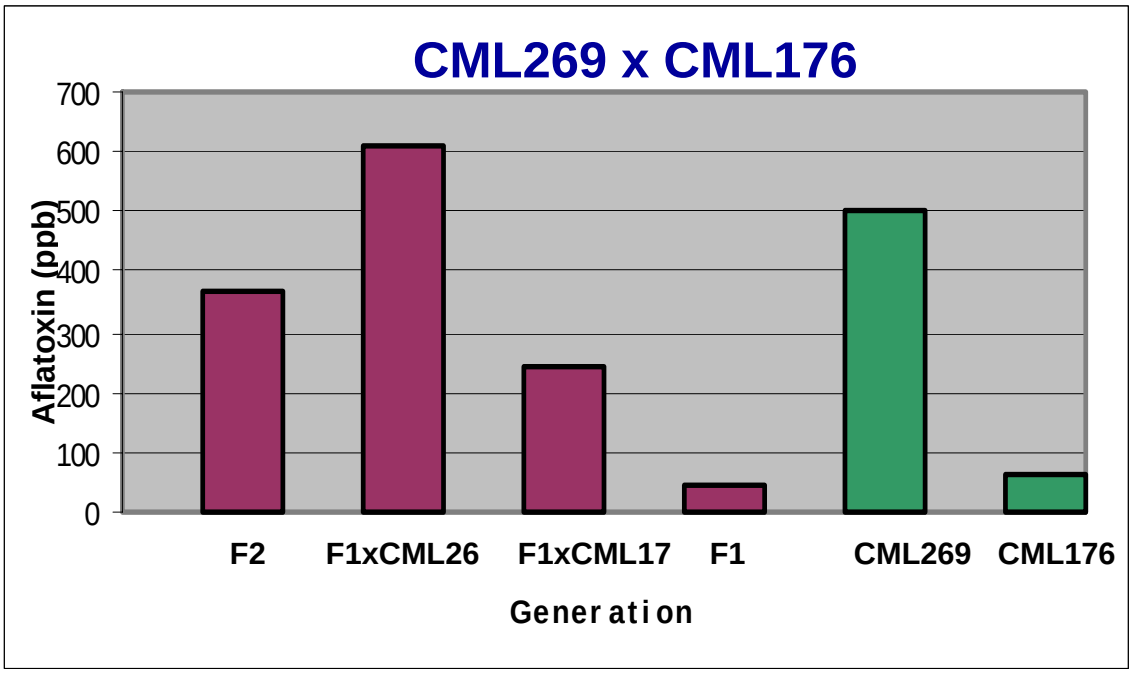


Figure 2.6. Means across locations (College Station and Weslaco, TX) for different generations of the cross between CML269 and CML176 in 2003

Discussion

Generation mean analysis is useful for identifying the types of gene action conditioning a trait, if both parents are significantly different and the genes for resistance are located in resistant parent.

The frequency distribution of aflatoxin in the six tests was generally skewed towards the resistant parents in all tests. In the 2003 trials, College Station recorded very high level of aflatoxin concentration. This is probably due to the rainy condition during flowering and high temperatures. Inbreds CML176, CML161 and CML269 have shown less susceptibility to aflatoxin than Tx811, Tx804 and Tx114 in previous evaluations (Atta et al., 2004). The highest levels of resistance to aflatoxin were in the CML269 by CML176 and CML161 by Tx804 crosses with CML161 and CML176. In the cross between Tx114 by CML176, additive and dominance gene actions seem to be of primary importance and this agrees with the earlier research on a cross between inbreds B73 and LB31 which showed additive and dominance gene actions to be significant (Campbell et al., 1997). In some other crosses, additive gene action was significant and this is similar to findings by Hamblin and White (2000) that additive gene action is primarily responsible for aflatoxin resistance. Epistatic and additive gene actions were most important for reducing aflatoxin concentration in grain in the cross B73 x C12 (Walker and White, 2001).

The magnitude and relative importance of the gene action effects depends on the cross being investigated and the location. Environmental variation caused a great deal of difference in the response of individual crosses to aflatoxin accumulation. Additive gene effects were of primary in the following crosses, Tx114 by CML 176, Tx114 by CML269, and CML 269 by CML 176. The additive nature of the resistance to aflatoxin was reported by (Campbell et al., 1997). Dominance and dominance gene actions were significant in crosses Tx811 by CML176, Tx114 by CML176, and Tx804 by CML161.

The inbreds CML176 and CML161 can be used as sources of resistance in breeding programs and use for selection and identifying quantitative trait loci (QTLs).

In most of the cases, backcrosses to the most resistant parent were less susceptible than backcrosses to the susceptible parents. This is further evidence of the presence of resistance genetic factors in these lines.

GMA was effective in characterizing the mode of inheritance of aflatoxin response in these parental inbreds and their generations. The information obtained from this research would be useful in developing appropriate breeding procedures for transferring aflatoxin resistance from CML176 and CML161 into susceptible lines. This was used to select potential candidate lines and populations for genetic mapping of QTLs for aflatoxin resistance.

CHAPTER III

PHENOTYPIC CHARACTERIZATION OF A RECOMBINANT INBRED LINE MAPPING POPULATION CML161 X B73

Introduction

Maize (*Zea mays* L.) is ranked top among the world cereal crops in production and consumption (USDA – FAS 2003). In several developing countries in Africa, Asia and Latin America, maize is the staple food and most often the only source of protein in diet. Maize is frequently infected with fungi which produce toxins that affect the quality and safety of food and animal feeds. Two of the most serious corn contamination is due to *Aspergillus flavus* and *Fusarium verticilliodes*. Aflatoxin contamination is responsible for severe economic losses in corn in the South and Midwest (Widstrom, 1996). Aflatoxin is a potent toxin and carcinogen that can cause aflatoxicosis and liver cancer in animals and humans (Castegnaro and McGregor, 1998). Pre-harvest aflatoxin contamination during flowering and grain filling period is a major problem for maize growers worldwide. Aflatoxin contamination has been associated with abiotic stresses such as droughts and high temperature (Payne, 1992) and biotic stresses such as insect damage (Windham et al., 1999). In the US, maize with more than 20 ng g^{-1} of aflatoxin B1 is banned from interstate commerce while that with more than 300 ng g^{-1} cannot be used as livestock feed.

Cultural control practices such as using adapted varieties, irrigation, early planting dates and optimal fertilization can minimize aflatoxin contamination sometimes (Widstrom., 1996). However, no control strategy is completely effective when environmental conditions are extremely favorable for the growth of the fungus. The most desirable method of aflatoxin control is through host plant resistance to *A. flavus* infection. The limiting factors in breeding for aflatoxin resistance are the spatial and temporal variation in aflatoxin accumulation that requires inoculation and high number of replications, lack of a reliable and inexpensive screening methodology, and the low metabolic activity of

corn plants after physiological maturity (Payne, 1992). Aflatoxin resistance has been assumed to be a lowly heritable trait that is strongly influenced by environmental conditions. Understanding aflatoxin resistance both at the phenotypic and genotypic level is necessary for geneticists and breeders to develop efficient strategies for plant selection and introgression. For selection to be effective, the amount of genetic variance within the population, it should be a heritable trait (Bernardo, 2002).

One of the problems associated with aflatoxin is its high cost and time involved in its quantification. Because of this,, traits that are genetically and phenotypically correlated to aflatoxin tolerance could be used as an indirect selection for aflatoxin accumulation. It is also important that this secondary trait must have high heritability. One method used to measure the heritability of a trait is a recombinant inbred line RIL mapping population This RIL population can be used to estimate phenotypic and genotypic characteristics heritabilites, and to map QTLs for relevant traits. The objectives of this research were:

- To measure relevant phenotypic characteristics such as aflatoxin accumulation, days to silking, endosperm texture, percentage of rotten ears and grain yield in the population of RILs derived from the cross CML161 x B73*o2* including parental lines.
- To estimate heritability for each trait and their correlations to aflatoxin accumulation.
- To estimate correlation and relationship between aflatoxin accumulation and secondary traits in this RIL population.

Review of Literature

Overview

Aflatoxin is a naturally occurring toxin produced by the fungus *Aspergillus flavus*, which was recognized as a pathogen on maize in 1920. The toxin is the most potent carcinogen found in nature and confirmed to be the cause of human liver cancer (Castegnaro and Mc Gregor, 1998). The most commonly produced aflatoxin *A. flavus* are B1 and B2. *A. parasiticus* also produces two additional aflatoxins, G1 and G2. Although Aflatoxin B1 is the most studied in mammalian toxicology, it was recognized as a pathogen on maize in 1920 (Taubenhaus, 1920). Aflatoxin contamination of maize in the field has been associated with drought combined with high temperatures as well as insect injury (Payne, 1992).

High temperatures and drought stress increases the incidence of aflatoxin accumulation (Payne, 1998). High soil temperatures have also been found to increase aflatoxin concentration in maize (Megec et al., 1996) and in almonds (Doster and Michailides, 1995). Combination of high temperature and drought conditions during kernel filling increases aflatoxin accumulation (Lisker and Lillehoj, 1991; Vincelli et al., 1995). The US Food and Drug Administration (2000) set standards for the consumption of aflatoxin. Aflatoxin contamination of maize intended for food with more than 20 ng g^{-1} of aflatoxin B1 is banned from interstate commerce and that with more than 300 ng g^{-1} cannot be used as livestock feed.

Apart from drought stress and high temperatures, damage from insects feeding have been reported to be associated with higher aflatoxin accumulation (Windham et al.,1999) Insects such as corn earworm (*Helicoverpa zea*) and European corn borer (*Ostrinia nubilalis)* increased aflatoxin contamination by feeding on and damaging developing kernels and by transporting *A. flavus* conidia into the ear (McMillian,1983).

Management Strategies

Several management practices have been employed in the control of aflatoxin. Cultural control practices such as using adapted varieties, irrigation, early planting dates and optimal fertilization can minimize aflatoxin contamination most years (Widstrom, 1996). Controlling insect damage, timely harvesting and avoiding drought stress can contribute to aflatoxin reduction. Timely weed control can also reduce the incidence of aflatoxin by eliminating stress due to competition to the growing maize plant (Lillehoj, 1983). Chemical methods of resistance under genetic control have been identified. Huang et al. (1997) identified two kernel proteins that appear to confer resistance. Chen et al. (1998) also suggested trypsin inhibitor in kernels that confer resistance when available in high concentrations. Physical methods of aflatoxin resistance have been identified such as husk covering thicker pericarp layers may inhibit infection of *Aspergillus flavus* into the undamaged kernels (Tubajika and Damann, 2001). Traits such as husk covering and tightness and physical properties of the pericarp (thickness, wax) contribute to aflatoxin resistance (Betran et al., 2002).

More recent techniques such as biological control have been applied in the control of aflatoxin. Encouraging is the use of intraspecies competition between non-toxic strains of *Aspergillus* that live in the same ecological niche. Native aflatoxigenic strains effectively compete with *A. flavus* during the infection stage (Cotty, 1994; Dorner et al., 1998). However, no control strategy is completely effective when environmental conditions are extremely favorable for growth of the fungus. The most desirable method of aflatoxin control is through host plant resistance to the fungus.

Breeding Approach

Resistance to aflatoxin contamination is considered to be under genetic control and genetic variation for response to aflatoxin has been found in maize (Scott and Zummo, 1988; Campbell and White, 1995a). Several sources of resistance have been identified

that are resistant to *Aspergillus flavus* such as Mp420, Mp715 and Tex6 (Scott and Zummo, 1990; Campbell et al., 1993; White et al., 1997). Heritability determines the progress that can be made on selecting the desired breeding material. Heritability involves the ratio of genetic variance to the total phenotypic variance. Genetic variance is composed of additive, dominant and epistatic variance. Aflatoxin is considered to have low heritability due to the influence of genotype by environment interactions (Hamblin and White, 2000).

Widstrom et al. (1987) listed four major genetically controlled traits that could influence or condition resistance to aflatoxin contamination namely resistance to infection process, resistance to aflatoxin formation once infection has occurred, resistance to insect damage, and resistance to environmental stress. One way of determining the heritability of a trait is the use of mapping populations. There are several forms of mapping populations such as near isogenic lines, double haploids, recombinant inbred lines and backcross populations. The most popularly used is the recombinant inbred line. It involves hybridizing two inbreds that contrasts in different agronomic characteristics. It gives estimates of the additive genetic variance since it can be evaluated in different environments and years.

Materials and Methods

Germplasm and Environments

A mapping population composed of 300 recombinant inbred lines (RIL) was derived from a cross between CML161 and B73. Inbred CML161 is an exotic subtropical inbred classified as Quality Protein Maize (QPM) and released by CIMMYT. It has flint endosperm and white cobs. B73*o2* is a floury B73 carrying a non-functional mutation in the *opaque-2* gene, which regulates the expression of alpha zeins. The two parents, CML161 and B73*o2*, differ in several agronomic characteristic such as flowering dates and susceptibility to pests and diseases. The two inbreds were crossed and selfed continuously for six generations to produce the S5 mapping population. The mapping

population was evaluated in two Texas locations, Weslaco (latitude $26^{o}09$, elevation 22-5m) and College Station (latitude $30^{o}37$, elevation 96m). Single row plots were 5.079 m^2 in College Station and 4.047 m^2 in Weslaco. The field experimental design was alpha lattice with three replications in Weslaco and two replications in College Station.

Phenotypic Evaluation

The traits were inoculated with *Aspergillus flavus* isolate NRRL3357. The method of inoculation used was the non-wounding silk channel inoculation technique (Zummo and Scott, 1989). The plants were inoculated six to ten days after midsilk using a conidial suspension containing 3 x 10^7 conidia of *A. flavus* in 3 ml of distilled water injected into the plant. Data were recorded on plot basis for all experiments on the following agronomic traits:

- Aflatoxin concentration: the whole plot grain sample was ground using a Romer mill and 50g sub samples of the flour were used to quantify aflatoxin using monoclonal antibody affinity columns and fluorescence determination using the Vicam Aflatest (Watertown, MA). It is expressed in parts per billion (ppb or ng g^{-1}).
- Maturity: number of days from planting until the day when 50% of the plants showing silks.
- Endosperm texture: visual rating of kernel in a scale 1 to 5 where 1= flinty endosperm having round crown kernel vitreous appearance and 5 = soft endosperm with marked dentation.
- Percentage of rotten ears: measured as percentage of the number of diseased ears divided by the number of inoculated ears.
- Grain yield per ear: it was estimated by dividing the total plot grain weight over the number of ears. It was reported in grams per ear.

Statistical Analysis

Aflatoxin concentrations (measured in ppb) were transformed by the logarithm to normalize the variance. Analysis for individual environments were completed using REMLtool (Welen, 2003) as an alpha lattice with and without spatial analysis and randomized complete block design. Adjusted means were obtained using the method with the lowest mean square error. Across environments analysis was conducted using PROC GLM in SAS 9.0 (SAS Institute, 2002). The genotypes, replications and environment were considered random. Variance components and heritability estimates were obtained using SAS procedures (Holland et al., 2003). Family basis heritability estimates were obtained for single and combined environments.

For single environment heritability estimates were calculated as:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{r}}$$

Where,

σ_g^2 = the genotype variance,

σ_e^2 = is the error variance, and

r = the number of replications.

For across environments heritability estimates were calculated as:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{e} + \frac{\sigma_e^2}{r'e}}$$

where,

σ_g^2 and σ_e^2 are the same as above, while σ_{ge}^2 is the genotype x environment interaction.

Pearson correlation was computed between aflatoxin and related traits for individual and across locations as:

$$r = \frac{\sum (x - \bar{x})(y - \bar{y})}{\sqrt{\sum (x - \bar{x})^2 \sum (y - \bar{y})^2}}$$

where, *x* and *y* represent traits X and Y.

Biplots using single value decomposition or principal component analysis were constructed for individual environments and across environments using standardized value for aflatoxin and secondary traits. These biplots illustrate the relationship among the traits. Biplot were constructed using macros in MS Excel

Biplot v1.1 (Dr. E.P. Smith, Virginia Tech; http://www.stat.vt.edu/facstaff/epsmith.html).

Results

Aflatoxin Accumulation

Significant differences were detected for aflatoxin concentration in both locations. Aflatoxin accumulation for this mapping population in Weslaco, TX averaged 1161.92 ng g^{-1} and reached a maximum of 4949 ng g^{-1}. In College Station, TX aflatoxin accumulation averaged 2041.84 ng g^{-1} (Table 3.1). Across environments, aflatoxin concentration averaged 1605.37 ng g^{-1}. These levels of aflatoxin concentration for both of these locations are relatively higher when compared to other studies (Betran et al., 2002; Scott and Zummo, 1998).

The logarithmic transformation of aflatoxin accumulation for the mapping population in Weslaco averaged 2.85, in College Station 3.04, and across environments 2.94. Heritability estimate across environment was 0.40, which is intermediate between Weslaco (H = 0.54) and College Station (H = 0.33) (Table 3.2).

The distribution of aflatoxin (ng g^{-1}) was skewed at both locations (Figure 3.1) but was normal with the logarithmic transformation (Figure 3.2). In both trials, B73o2 has higher mean aflatoxin concentration than CML 161 (Tables 2.1, 2.2) and highly significant genotype by environment interactions. The heritability estimates were higher in Weslaco than in College Station. Higher aflatoxin values and more favorable experimental

conditions to induce aflatoxin contamination in Weslaco could explain the observed difference between the two locations. Heritability measures the portion of phenotypic variance that is due to genetic effect when the heritability estimate is high across environment. It indicates that genetics plays a major role in the phenotype.

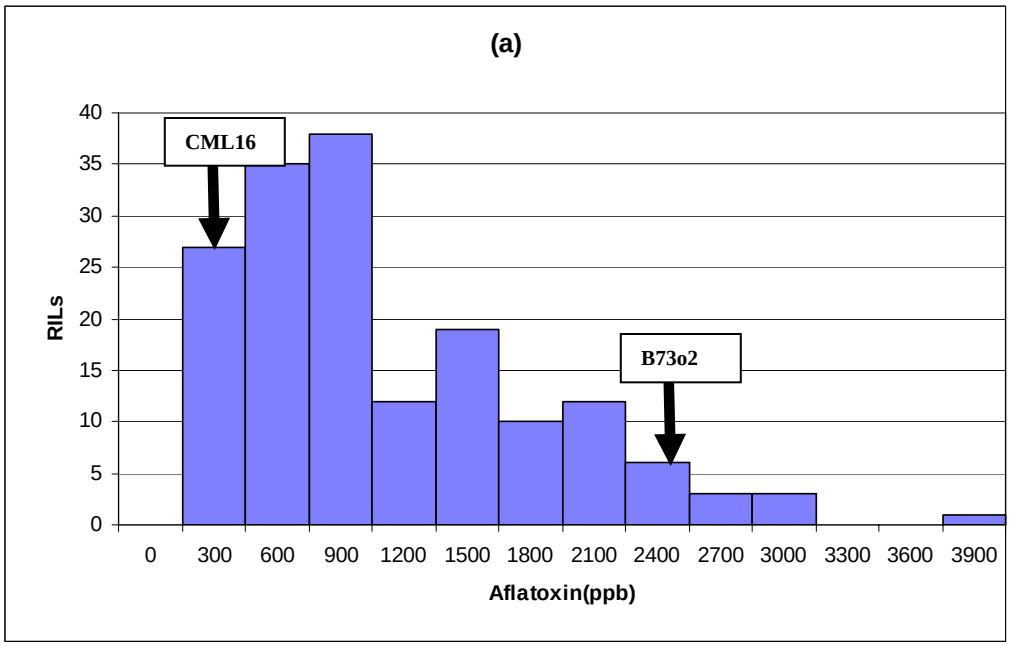
Table 3.1. Statistical information and analysis of variance components and heritabilites for aflatoxin concentration (ng g^{-1}) for CML161 by B73*o2* mapping population per and across environments in 2005.

	Weslaco	College Station	Across Environment
Mean CML161	417.78	506.77	462.27
Mean B73o2	2581.95	4658.63	3620.28
Overall mean	1161.92	2041.84	1605.37
Range	4900.00	8092.06	4713.54
Rep	0.0191	0.0928	0.0518
Genotype	0.0001	0.0172	0.0001
Environment	NA	NA	0.003
Genotype x environment interaction	NA	NA	0.0001
Heritability	0.53 ± 0.06	0.44 ± 0.07	0.52 ± 0.07

Table 3.2. Statistical information and analysis of variance components and heritabilites for aflatoxin concentration (log) for CML 161 by B73*o2* mapping population per and across environments in 2005.

	Weslaco	College Station	Across Environment
Mean CML161	2.34	2.13	2.24
Mean B73o2	3.34	3.58	3.46
Overall Mean	2.85	3.04	2.94
Range	2.06	2.59	1.62
Rep	0.0001	0.8304	0.0001
Genotype	0.0001	0.0003	0.0001
Environment	NA	NA	0.0001
Genotype x environment interaction	NA	NA	0.0123
Heritability	0.54 ± 0.06	0.33 ± 0.10	0.40 ± 0.09

± Standard Error



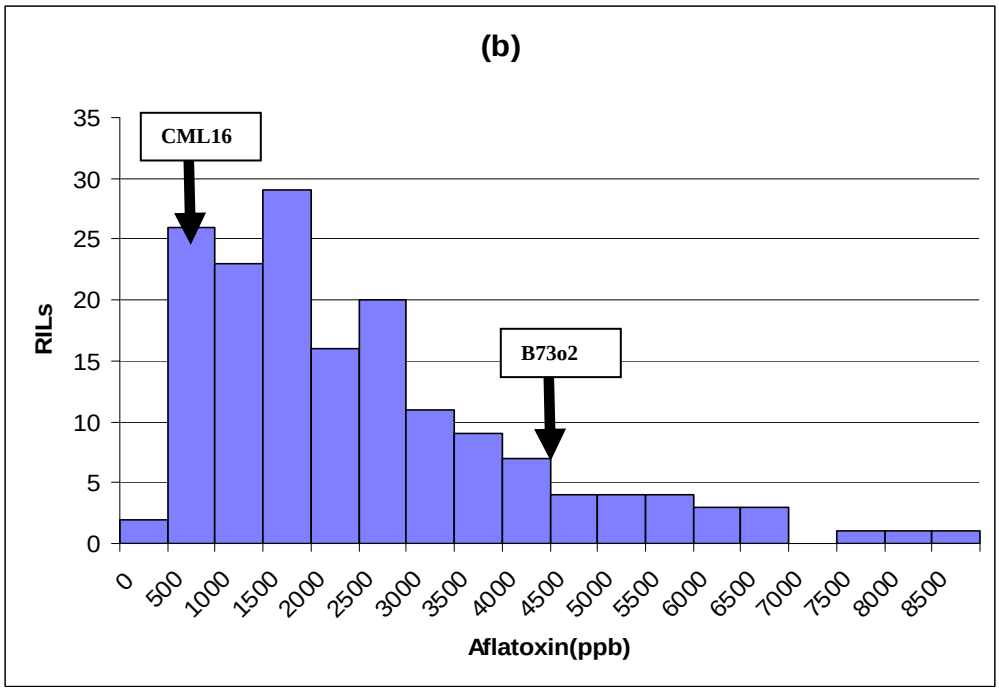


Figure 3.1 Frequency distributions of RIL population for aflatoxin accumulation measured in parts per billion in Weslaco (a) and in College Station (b). Arrows indicate mean concentration for parental inbred lines CML161 and B73o2.

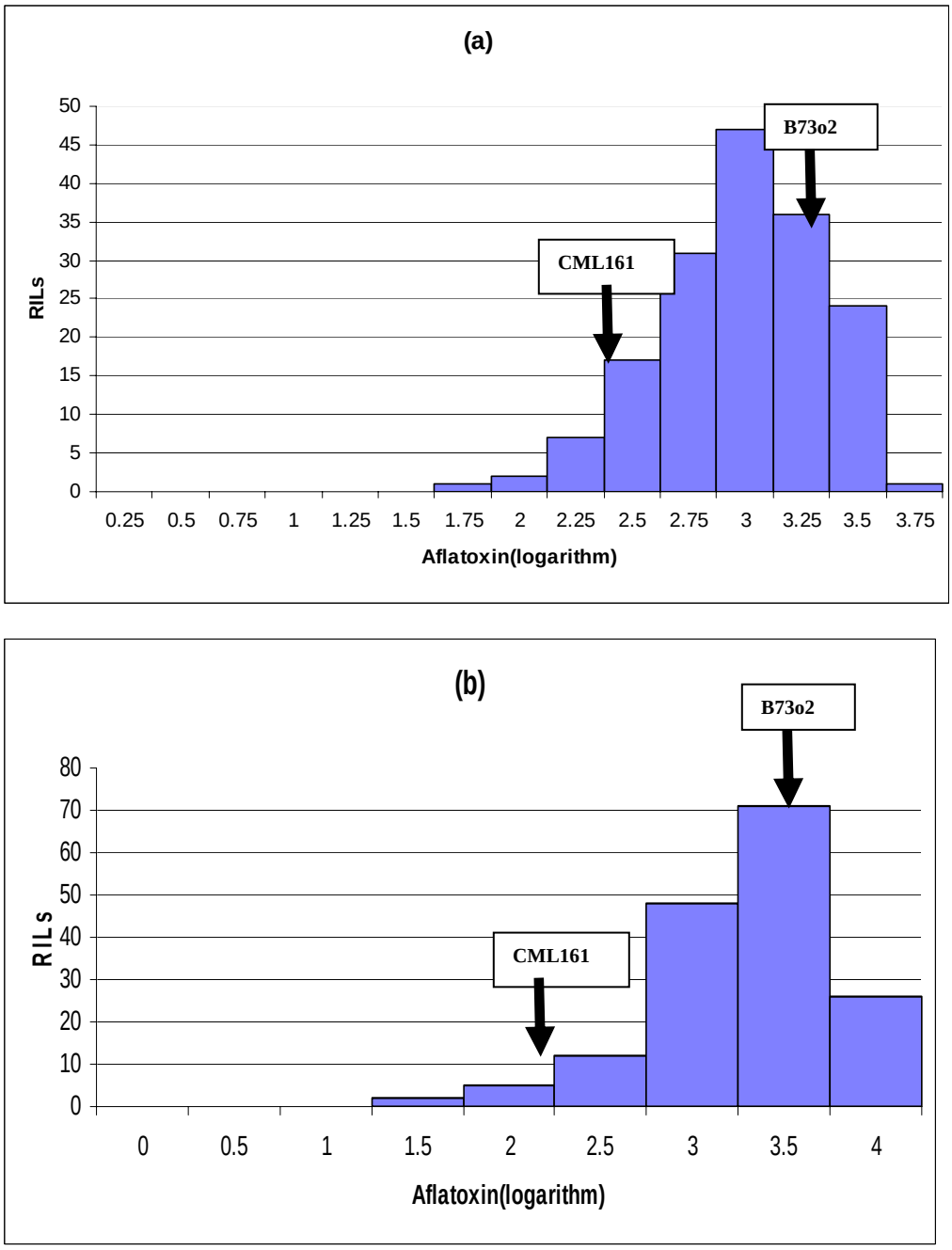


Figure 3.2 Frequency distribution of RIL population for aflatoxin accumulation measured as logarithmic transformation in Weslaco (a) and in College Station (b) in 2005.Arrows indicate mean concentration for parental inbred lines CML161 and B73o2

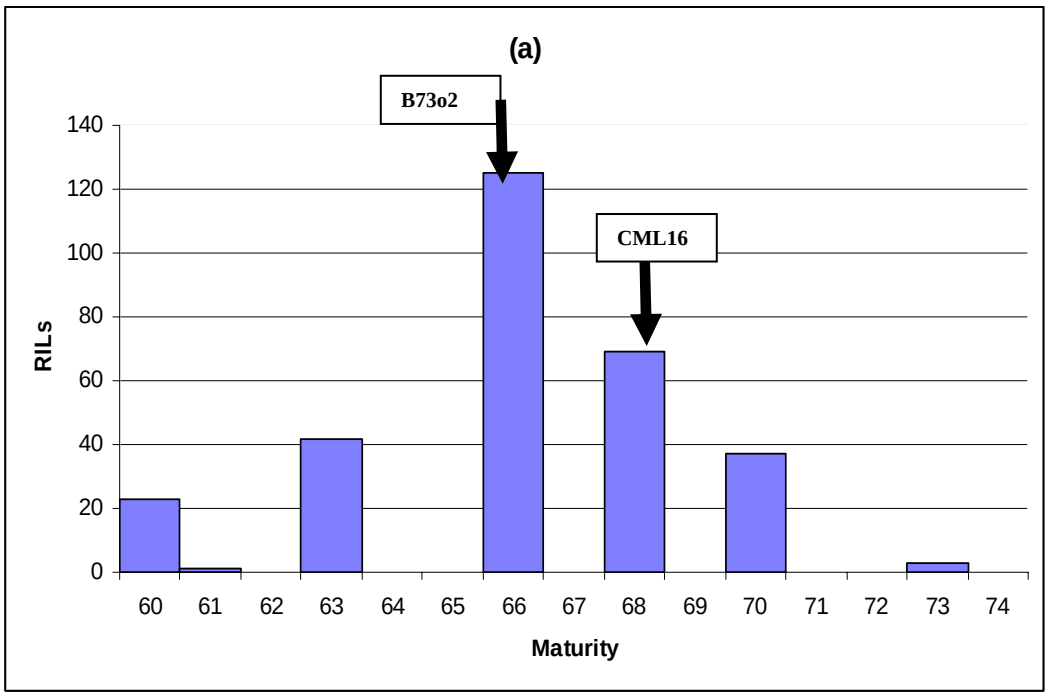
Maturity

There were significant differences for female flowering in the two environments. Parental line CML161 flowered later (68 days) than B73o2 (66 days) in Weslaco (Table 3.3). Heritability estimates for Weslaco was high (H = 0.99). In Weslaco B73*o2* had higher mean level of aflatoxin concentration and was more susceptible to pests and diseases. Aflatoxin concentration is high during drought and high temperature when early flowering material coincides with kernel filling stage under high temperature. It leads to increase in aflatoxin contamination. This could be a possible explanation why parental line B73*o2* had higher concentration of aflatoxin when compared to CML161. Days to 50% silking showed transgressive segregation in both locations (Figure. 3.3).

Table 3.3. Statistical information and analysis of variance components and heritabilites for maturity (days to silking) for CML161 x B73o2.

	Weslaco	College Station	Across Environment
Mean CML 161	68.00	69.40	69.11
Mean B 73o2	66.83	70.29	69.22
Overall Mean	61.68	70.00	68.70
Range	10.00	15.56	14.33
Rep	0.0198	0.0001	0.0001
Genotype	0.0051	0.8355	0.0001
Environment	NA	NA	0.0014
Genotype x environment interaction	NA	NA	0.0001
Heritability	0.99 ± 0.00	0.45 ± 0	0 ± 0

±= Standard Error



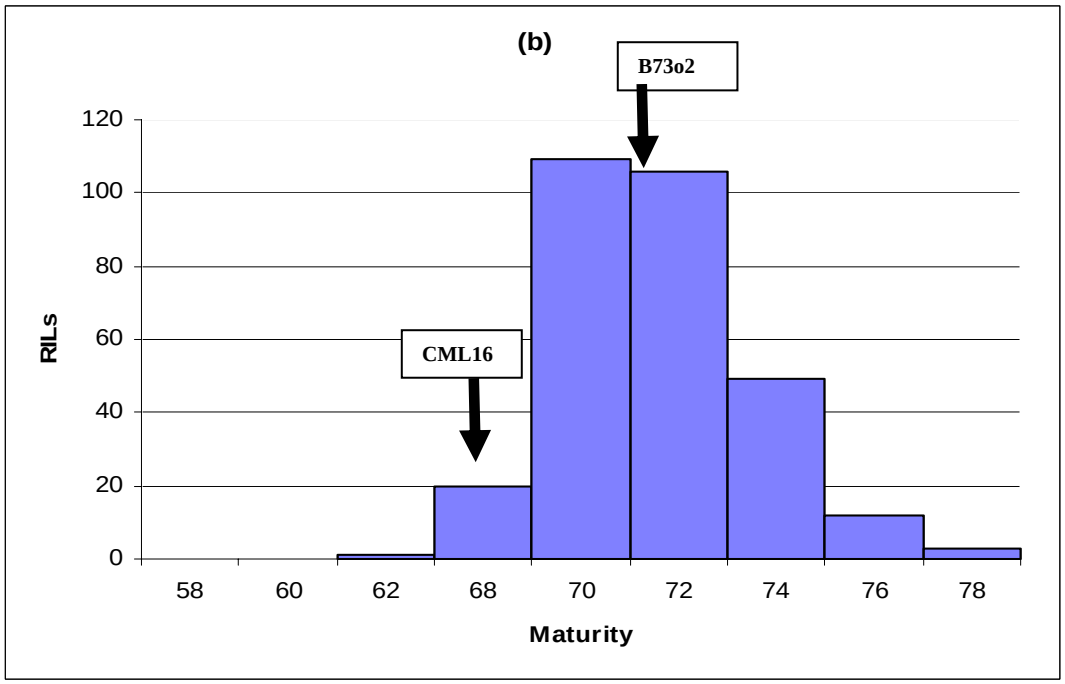


Figure 3.3 Frequency distribution of RIL population for maturity measured as 50% days to silking in Weslaco (a) and in College Station (b) in 2005. Arrows indicate mean concentration for parental inbred lines CML161 and B73*o2*.

Percentage of Rotten Ears

There was significant difference between the parental lines and environments for the percentage of rotten ears. College Station had higher average percentage of rotten ears (38.73) compared to Weslaco (20.08) (Table 3.4). Also the parental line CML161 had low percentage of rotten ears while B73*o2* had a much higher percentage of rotten ears in both and across location. These findings confirm earlier findings that B73*o2* is more susceptible to pests and diseases. There was significant genotype by environment interaction. Heritability estimate was higher for Weslaco (H = 0.68), intermediate for College Station (H = 0.47) and across locations (H = 0.49). The distribution of percentage of rotten ears is skewed (Figure 3.4) in both locations. The intermediate to high heritability for this trait makes it justifiable to be selected for in early generation.

Table 3 4. Statistical information and analysis of variance components and heritabilites for percentage of rotten ears for CML161 x B73*o2* mapping population per and across environments in 2005.

	Weslaco	College Station	Across Environment
Mean CML 161	0.00	2.08	0.83
Mean B 73	39.73	64.59	49.89
Overall Mean	20.08	38.08	27.51
Range	100.00	100.00	93.75
Rep	0.5091	0.0461	0.097
Genotype	0.0001	0.0001	0.0001
Environment	NA	NA	0.0001
Genotype x environment interaction	NA	NA	0.0001
Heritability	0.68 ± 0.03	0.47 ± 0.06	0.49 ± 0.00

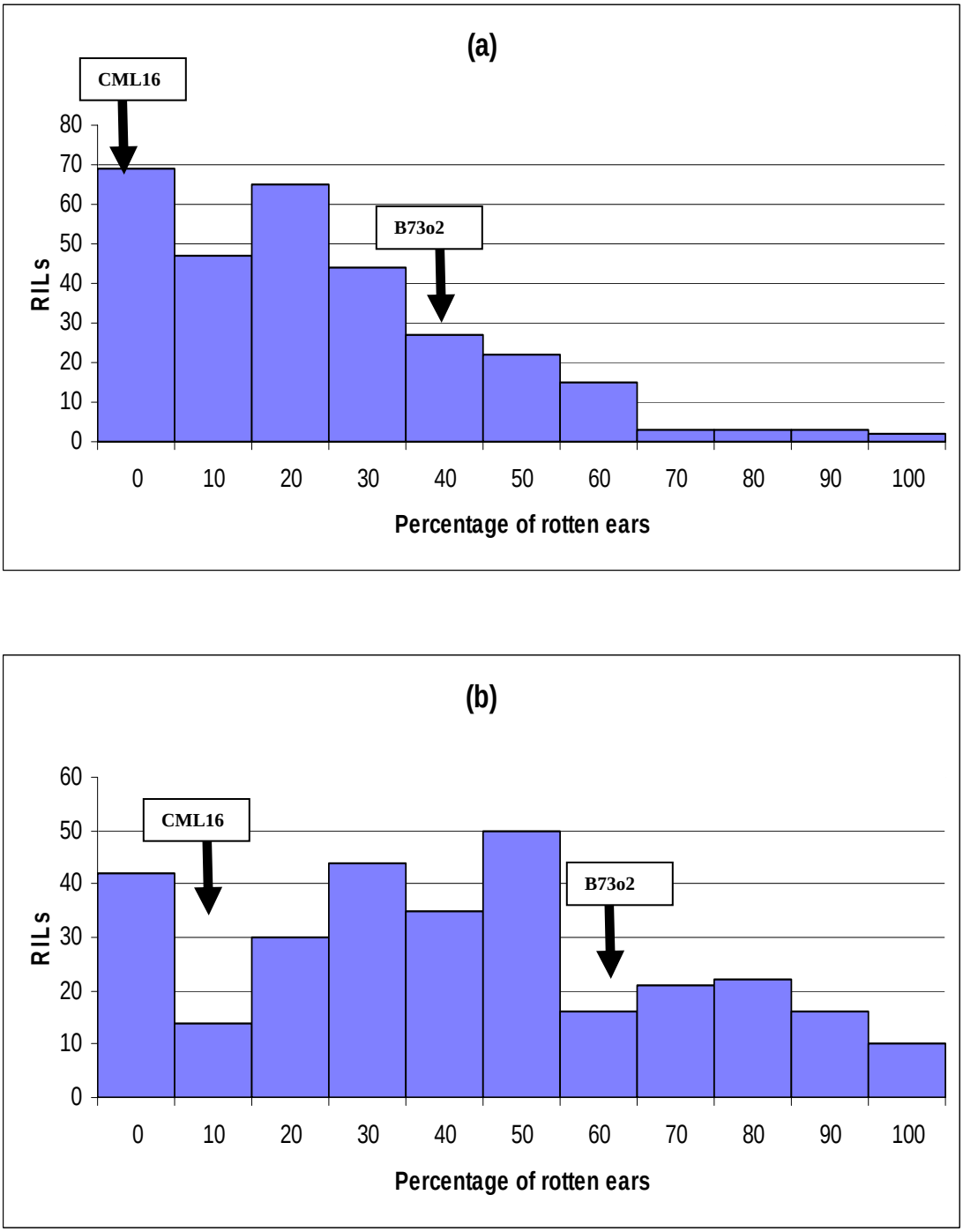


Figure 3.4 Frequency distribution of RIL population for percentage of rotten ears in Weslaco (a) and in College Station (b). Arrows indicate mean concentration for parental inbred lines CML161 and B73*o2*.

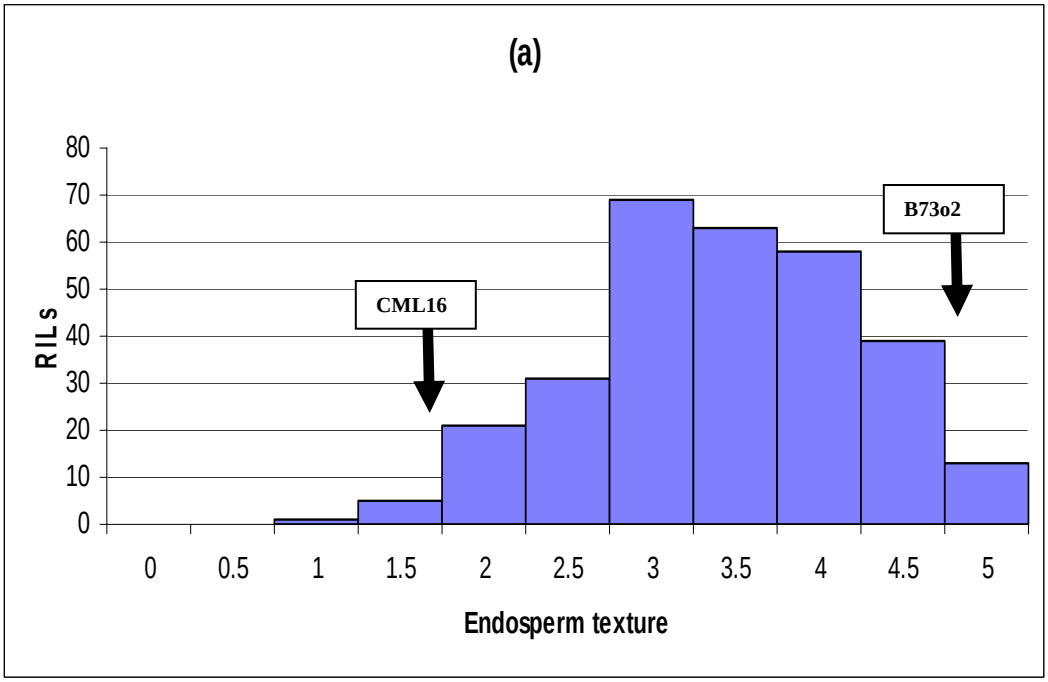
Endosperm Texture

There were significant differences between the parental means in both and across locations. B73*o2* had higher rating than CML161; hence, it is more floury while CML161 is more flinty (Table 3.5). There were no significant differences among the means for the parental inbred in both locations. For example B73*o2* had a mean of 4.91 in Weslaco and in College Station 4.47. There were also no significant differences in the overall mean between both locations and across locations. Flinty endosperm is affiliated with lower aflatoxin levels (Figure 3.5).

The heritability were significantly high for Weslaco (H = 0.81), College Station (H = 0.74) and across location (H = 0.83). This high heritability makes it easily selectable during early stage of breeding. There was high significant variation among genotypes for both environments and across environments. Genotype by environment interaction was also significant.

Table 3.5. Statistical information and analysis of variance components and heritabilites for endosperm texture for CML 161 x B73*o2* mapping population per and across environments in 2005.

	Weslaco	College Station	Across Environment
Mean CML 161	1.68	1.07	1.37
Mean B 73	4.83	4.47	4.66
Overall Mean	3.24	3.11	3.18
Range	4.00	4.07	4.00
Rep	0.8656	0.0006	0.0027
Genotype	0.0001	0.0001	0.0001
Environment	NA	NA	0.0003
Genotype x environment interaction	NA	NA	0.0099
Heritability	0.81 ± 0.01	0.65 ± 0.04	0.83 ± 0.02



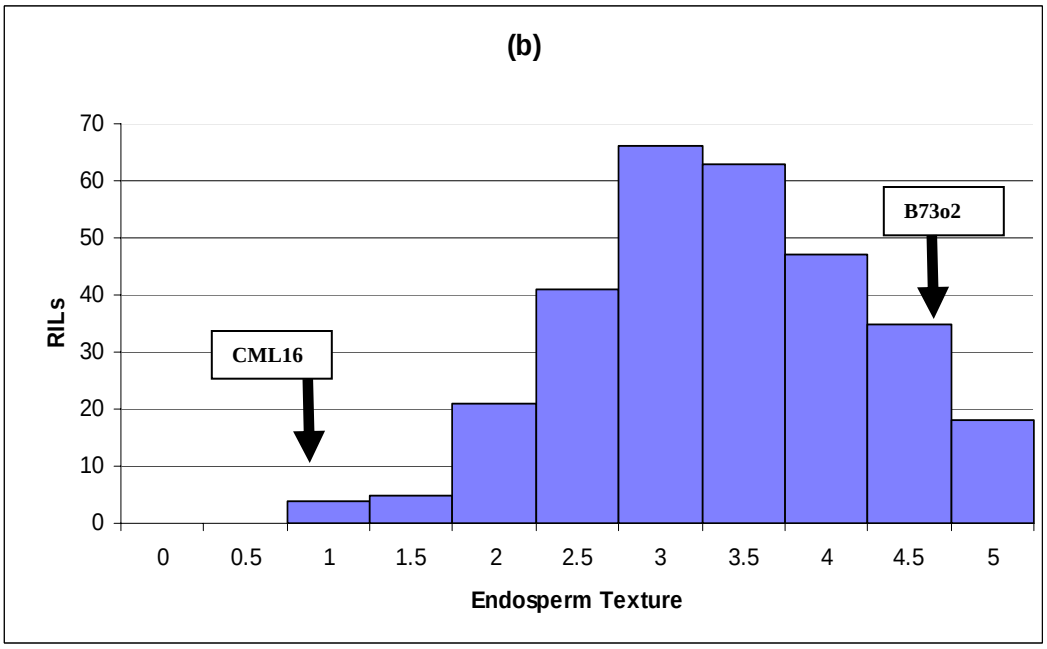


Figure 3.5 Frequency distribution of RIL population for endosperm texture scored as 1=flinty and 5=floury in Weslaco (a) and in College Station (b) in 2005. Arrows indicate mean concentration for parental inbred lines CML161 and B73*o2*.

Grain Yield

There was significant difference among the parental inbreds in both locations and across locations. Grain yield for CML 161 was significantly higher than yield for B73*o2* (Table 3.6). There was transgressive segregation in the distribution of grain yield, which was normally distributed in both locations (Figure 3.6). Grain yield was highly heritable in Weslaco (H = 0.81), and intermediate levels of heritability in College Station (H = 0.65) and across locations (H = 0.54). Genotype by environment interaction was highly significant across both locations.

Table 3.6. Statistical information and analysis of variance components and heritabilites for grain yield (grams per ear) for CML161 by B73*o2* mapping population per and across environments in 2005.

	Weslaco	College Station	Across Environment
Mean CML 161	31.05	23.20	22.86
Mean B 73	17.69	9.49	17.58
Ovearall Mean	24.02	22.60	23.61
Range	54.40	62.80	49.28
Rep	0.4118	0.0001	0.0001
Genotype	0.0001	0.0001	0.0001
Environment	NA	NA	0.0123
Genotype x environment interaction	NA	NA	0.0001
Heritability	0.81 ± 0.01	0.65 ± 0.04	0.54 ± 0.05

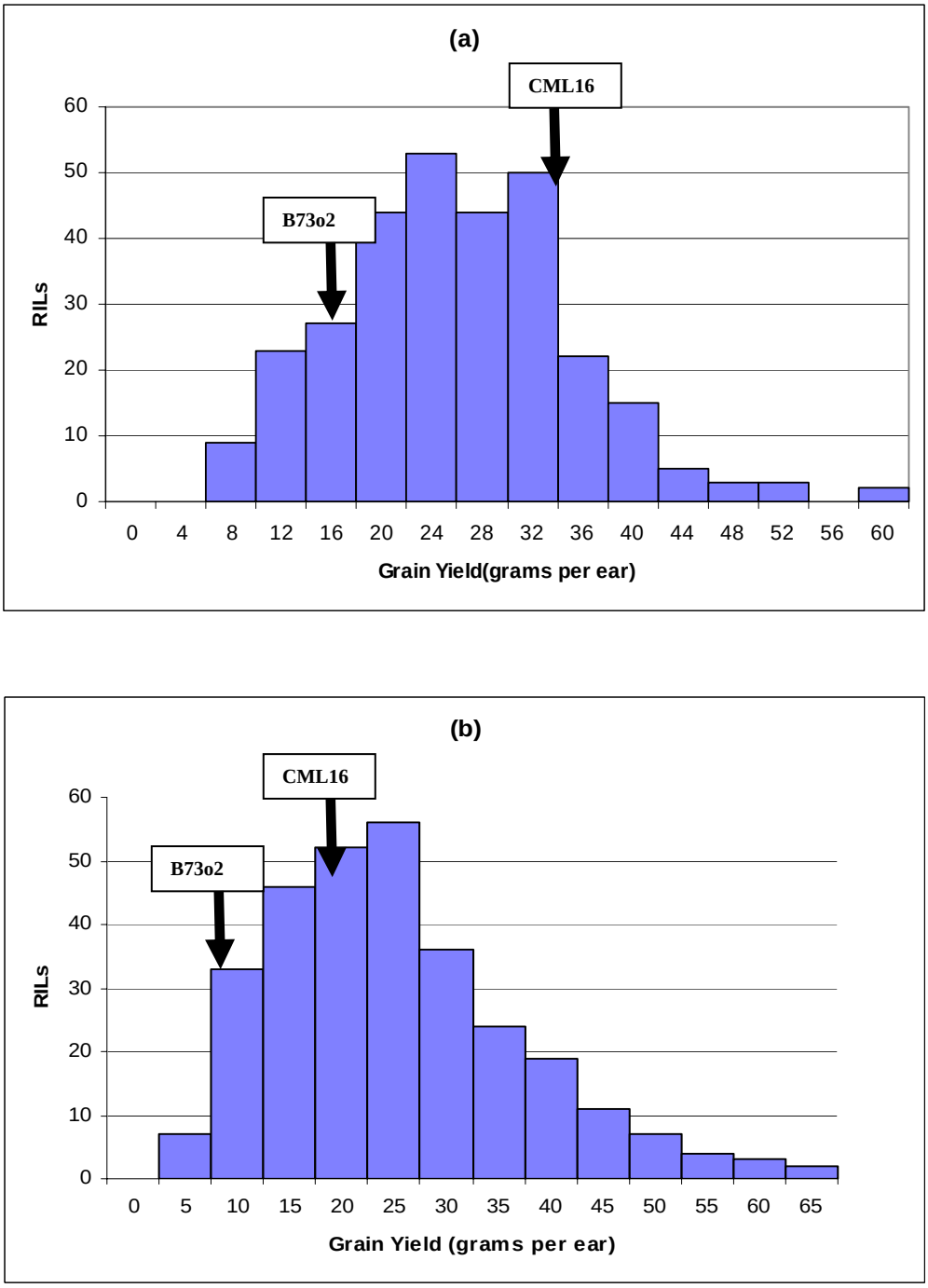


Figure 3.6 Frequency Distribution of RIL population for grain yield measured in grams per ear in Weslaco (a) and in College Station (b) in 2005. Arrows indicate mean concentration for parental inbred lines CML161 and B73*o2*.

Trait Correlations

Correlation between traits was determined using Pearson correlation coefficient and genotypic and phenotypic correlations. Estimates of Pearson correlations among traits for Weslaco, College Station, and across both locations are presented in Tables 3.7, 3.9 and 3.11, respectively. Genotypic and phenotypic correlations between aflatoxin and other traits for Weslaco, College Station and across both locations were also illustrated in Figures 3.8, 3.10 and 3.12, respectively.

Results from Pearson's and genotypic and phenotypic correlations produced similar results. At Weslaco, aflatoxin concentration was positively correlated with percentage of rotten ears (r = 0.35) and negatively correlated to grain yield per ear (r = -0.25). Aflatoxin concentration (ppb) was genotypically and positively correlated to percentage of rotten ears (rg = 0.73**) and negatively correlated to grain yield (r_g = -0.33) (Tables 3.7 and 3.8).

In College Station, aflatoxin was positively correlated to percentage of rotten ears (r = 0.30 and negatively correlated to grain yield (r = -0.27). And was also genotypically correlated to percentage of rotten ears (r_g = 0.41**). (Tables 3.9 and 3.10). Similar results were observed for across location analysis.

Singular value decomposition biplots were used to illustrate trait correlation in individual and across environment (Figures 3.7, 3.8 and 3.9)

Table 3.7. Pearson's correlations among phenotypic traits in Weslaco for CML161 x B73*o2* recombinant inbred line population in 2005.

	afppb	ff	% rotten ears	gwt	gtrt
afppb	1.00				
ff	0.14	1.00			
%rottenears	0.36**	-0.15	1.00		
gwt	-0.26**	-0.87**	-0.06*	1.00	
gtrt	0.17**	0.59**	0.10**	-0.32**	1.00

**** Significant at 0.01 and * at 0.05**

afppb=Aflatoxin(ng g^{-1}), ff=Female flowering ,% rotten ears=Percentage of rotten ears, gwt=Grain yield(grams), gtrt=Endosperm texture (ratings 1=flint to 5=dent).

Table 3. 8. Genotypic and phenotypic correlations between aflatoxin concentration in (ppb and logarithm) and secondary traits at Weslaco in 2005.

		Genotypic Correlations	**Phenotypic Correlations**
Maturity	AF	0.08 ± 0.111	0.04 ± 0.066
	Log AF	0.23 ± 0.108	0.16 ± 0.067
% rotten ears	AF	0.73 ± 0.132**	0.30 ± 0.047
	Log AF	0.91 ± 0.120**	0.39 ± 0.047
Grain yield	AF	- 0.37 ± 0.146**	- 0.17 ± 0.049
	Log AF	- 0. 15 ± 0.141**	- 0.02 ± 0.050
Endosperm texture	AF	0.27 ± 17.69	0.12± 7.627
	Log AF	0.52 ± 0.111**	0.26± 0.046

**** Significant at 0.01 and * at 0.05**

AF=Aflatoxin (ng g^{-1}), LogAF=Logaritmic transformation of Aflatoxin

Table 3. 9. Pearson's correlations among phenotypic traits in College Station for CML161 x B73o2 recombinant inbred line population in 2005.

	afppb	ff	% Rotten ears	gwt	gtrt
afppb	1.00				
ff	0.06**	1.00			
rottenears	0.31**	-0.03	1.00		
gwt	-0.27**	-0.20*	-0.02	1.00	
gtrt	0.18*	0.03	0.26**	0.02	1.00

**** Significant at 0.01 and * at 0.05**

Afppb=Aflatoxin(ng g^{-1}), ff=Female flowering ,% rotten ears=Percentage of rotten ears, gwt=Grain yield(grams), gtrt=Endosperm texture (ratings 1=flint to 5=dent).

Table 3.10. Genotypic and phenotypic correlations between aflatoxin concentration and secondary traits at College Station in 2005.

	Traits	Genotypic Correlations	Phenotypic Correlations
Ear to fungus ratio	AF	0.41 ± 0.20**	0.31 ± 0.05
	Log AF	0.68 ± 0.15**	0.43 ± 0.04
Grain yield	AF	– 0.24 ± 0.18	– 0.26 ± 0.05
	Log AF	– 0.11 ± 0.16	– 0.19 ± 0.05
Endosperm texture	AF	0.13 ± 23.12	0.16 ± 16.081
	Log AF	0.25 ± 0.14	0.19 ± 0.05

**** Significant at 0.01 and * at 0.05**

AF=Aflatoxin (ng g^{-1}), LogAF=Logaritmic transformation of Aflatoxin

Table 3.11. Pearson's correlations among phenotypic traits across locations for CML161 x B73*o2* recombinant inbred line population in 2005.

	afppb	ff	% rotten ears	gwt	gtrt
afppb	1.00				
ff	0.14**	1.00			
rottenears	0.35**	0.13	1.00		
gwt	-0.25**	-0.13**	-0.13**	1.00	
gtrt	0.13**	0.01	0.18	0.00	1.00

**** Significant at 0.01 and * at 0.05**

Afppb=Aflatoxin(ng g^{-1}), ff=Female flowering, %rottenears=Percentage of rotten ears, gwt=Grain yield(grams), gtrt=Endosperm texture (ratings 1=flint to 5=dent).

Table 3.12. Genotypic and phenotypic correlations between aflatoxin concentration and secondary traits across both locations in 2005.

	Traits	Genotypic Correlations	Phenotypic Correlations
Ear to fungus ratio	AF	1.22 ± 0.266**	0.30 ± 0.035
	Log AF	1.20 ± 0.219**	0.36 ± 0.033
Grain yield	AF	– 0.48 ± 0.226**	– 0.22 ± 0.037
	Log AF	– 0.57 ± 0.220	– 0.16 ± 0.039
Endosperm texture	AF	0.30 ± 4.916	0.13 ± 5.111
	Log AF	0.36 ± 0.147**	0.17 ± 0.037

**** Significant at 0.01 and * at 0.05**

AF=Aflatoxin (ng g^{-1}), LogAF=Logarithmic transformation of Aflatoxin

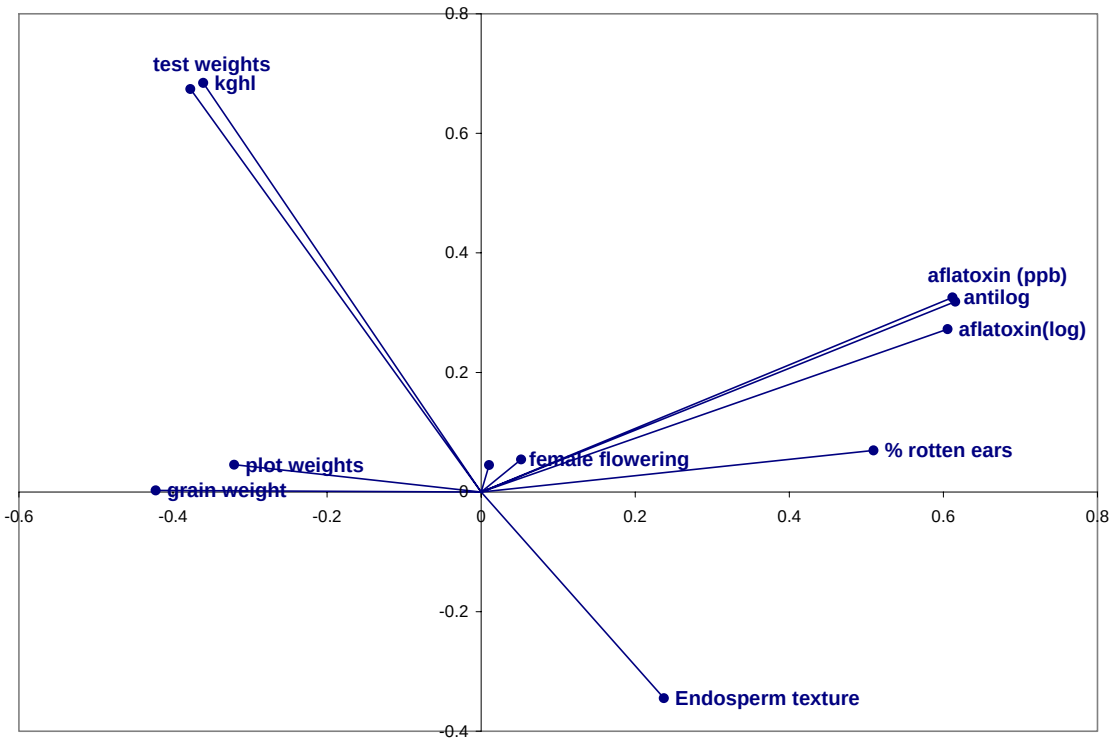


Figure 3.7. Single value decomposition biplot showing relationship among traits at Weslaco in 2005.

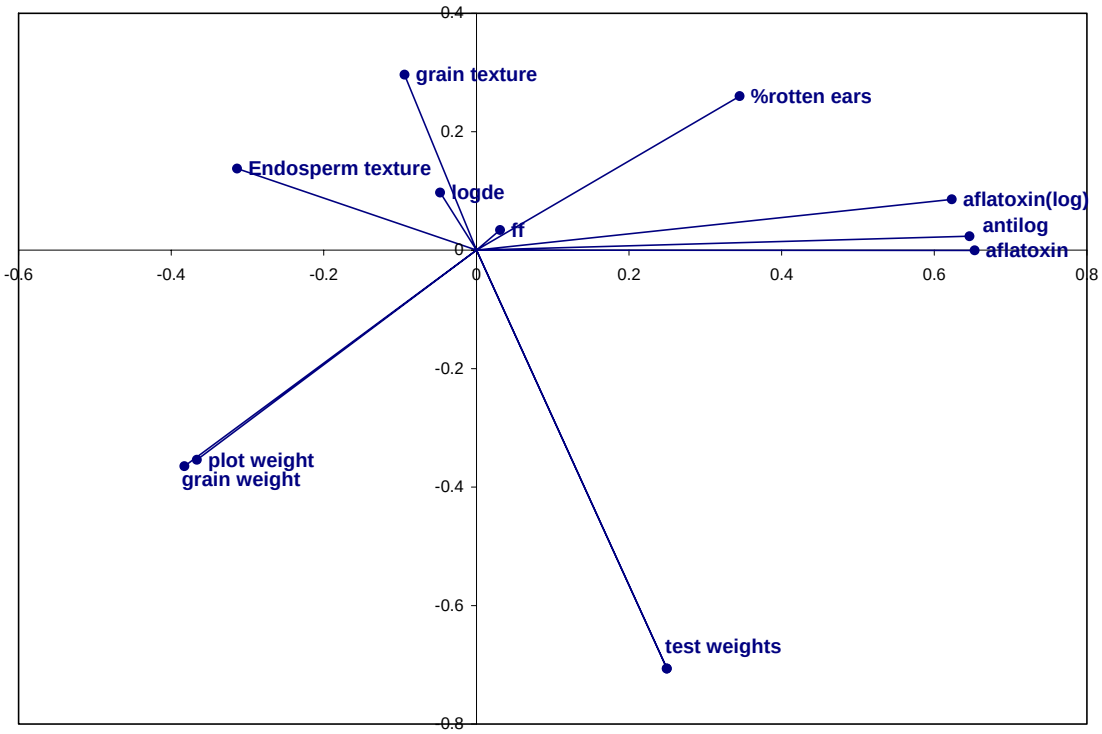


Figure 3.8. Single value decomposition biplot showing relationship among traits at College Station in 2005.

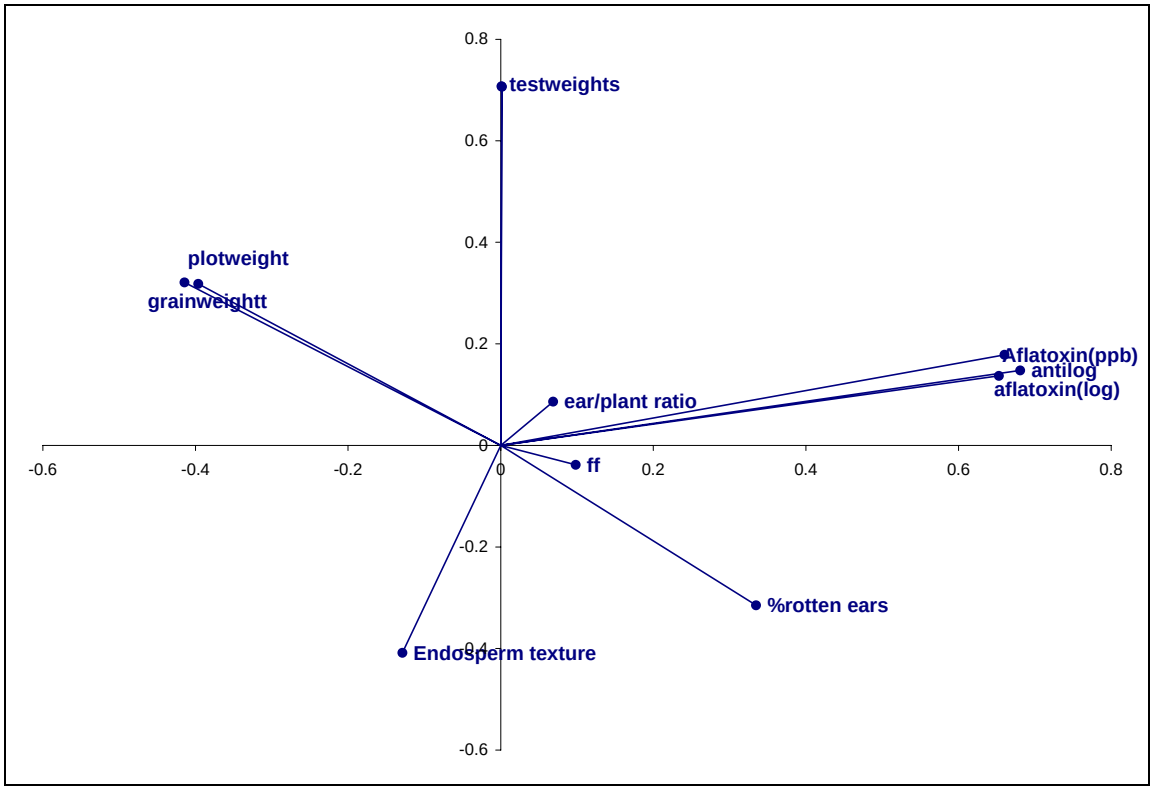


Figure 3.9. Single value decomposition biplot showing relationship among traits across locations in 2005.

Discussion

The incidence of aflatoxin contamination depends on the condition of the environment. High temperature favors the fungus as temperatures between 36^0C and 38^0C are optimum for the fungal growth (Payne, 1992). Analysis of variance across environments showed significant differences among the environments for all traits and significant differences among the two environments. Highly significant differences ($P > 0.001$) were observed among the RIL population in the individual environments and combined analysis. The level of aflatoxin concentration in Weslaco and College Station are higher when compared to previous studies (Betran et al., 2002; Scott and Zummo, 1998). In the 2005 trials for College Station, very high level of aflatoxin concentration was observed.
Heritability measures the portion of phenotypic variance that is due to genetic effect, it indicates that genetics plays a major role in the phenotype. The heritability estimates determines the amount of genetic gain from selection. These estimates are due to environmental and genotype by environment effects. For traits with low heritability multilocation testing is required in order to increase genetic gain. The heritability estimates for Weslaco and College Station were relatively high compared to earlier estimations (Betran et al., 2005).

In this study there was no significant correlation observed between maturity and aflatoxin accumulation. In the previous studies (Betran et al., 2006) observed a significant correlation between maturity and aflatoxin accumulation. Betran et al. (2004) reported that although correlation between maturity and aflatoxin was not significant, in general, early maturity hybrids had higher aflatoxin content than late maturity hybrids. B73*o2* was more susceptible to aflatoxin concentration than CML161. During grain filling if there is occurrence of drought and high stress it is likely to have high aflatoxin levels (Lisker and Lillehoj, 1991; Vincelli et al., 1995).

Aflatoxin is positively significantly correlated to percentage of rotten ears; this denotes that the higher the percentage of rotten ears the higher the aflatoxin accumulation. The

heritability estimates in Weslaco was quite high compared to other locations. The significant correlation with aflatoxin and its high heritability means that percentage of rotten ears could be used in indirect selection to reduce aflatoxin. Endosperm texture showed high heritability in each and across locations and significant correlations to aflatoxin. Flinty endosperm is associated with lower aflatoxin accumulation while soft or floury endosperm is more prone to attacks by pest and diseases such as aflatoxin contamination. Higher grain yield was associated with less aflatoxin contamination.
Betran et al. (2006) stated that correlation among traits can be due to pleiotrophy (same loci affecting both traits), linkage (different loci affecting the traits but traits are linked together), population structure (amount of linkage equilibrium) and environment effects.

Correlation and high heritability observed in this study can have strong breeding implications because it has the potential to increase genetic gain through selection indices and indirect selection (Falconer and Mackay, 1996). Indirect selection is effective when the secondary traits such as endosperm texture and percentage of rotten ears show a higher heritability than the primary trait. Secondly when there is significant correlation between both traits.

In conclusion, traits with moderate to high heritabilities and significant genotypic correlations to aflatoxin accumulations, could be used to indirectly select for aflatoxin resistance.

CHAPTER IV

GENETIC MAPPING AND ANALYSIS OF QUANTITATIVE TRAIT LOCI IN A MAIZE RECOMBINANT INBRED LINE POPULATION

Introduction

Aflatoxin is a potent toxin carcinogen produced by the fungus *Aspergillus flavus* and can cause aflatoxicosis and liver cancer in animals and humans (Castegnaro and Mc Gregor, 1998). Furthermore, aflatoxin contamination during flowering and grain filling period is a major problem in Texas. There are many techniques cultural practices such as early harvest, planting adapted cultivars and managing nutrient inputs that may help reduce the level of aflatoxin contamination in maize. Breeding for resistance to plant characteristics that reduce infection by aflatoxin accumulation is currently considered to be the most effective means to control contamination. Germplasm screening studies identified a number of public sources that have with lower levels of aflatoxin production; however, most of these sources of resistance have poor agronomic characteristics and this precludes their use in commercial hybrid production. Aflatoxin accumulation is influenced by environmental and genetic factors.

The most important step in breeding for resistance to aflatoxin accumulation is to identify sources of resistance that are agronomically desirable. Molecular breeding has proven in quite a few cases to be strategic for breeding resistant cultivars more efficiently. Robertson et al. (2005)proposes molecular breeding for aflatoxin production four reasons. First, when markers linked to resistance genes have been identified, the need to perform inoculations which are time consuming, labor intensive and expensive can be greatly reduced. Second, screening plants with markers associated with known resistance genes is more cost efficient than phenotypic evaluation mycotoxin levels each season throughout a breeding program because phenotypic evaluations require costly laboratory techniques. Third, selection for markers alleles linked to resistance genes can be performed in individual plants, while mycotoxin assays which usually require multiple plants and replications to obtain accurate data. Finally, marker assisted

breeding can be implemented in environments or in off season nurseries permitting multiple generations of selections each year and speeding up the development of a resistant cultivar. Resistance to aflatoxin accumulation is a quantitative trait, therefore it is logical to assume that aflatoxin resistance is controlled by multiple QTL. To identify QTL associated with a reduction in mycotoxin, a mapping population that is segregating for resistance must be developed.. To complete QTL mapping the population must be evaluated for the trait of interest and genotypic data for each individual in the population with a set of well distributed polymorphic markers, must be completed. Once completed a powerful statistical method to identify an association between both sets of data. Several types of mapping populations exist such as haploid, double haploid, recombinant inbred lines (RILs), F2, and backcross populations each with their relative strength and weakness. For the purpose of this research, RILs will be used because they are permanent mapping populations that can be used in long-term, multiple (locations and times) experiments. RILs are produced by continually selfing or sib-making the progeny of individual members of an F2 population until homozygosity is achieved. A RIL population for studying response to aflatoxin was developed using inbred lines CML 161 and B73*o2* as parents. These lines differ for several agronomic traits. The phenotypic data from several locations is combined with genotypic data to map aflatoxin resistant genes. The objectives of this research were:

- To identify QTL's response for the phenotypic variation of aflatoxin accumulation and its secondary traits.
- To identify if these QTLs were consistent in the two environments.

Review of Literature

Aflatoxin Overview

Aflatoxin B_1 is a mycotoxin produced by the fungus *Aspergillus flavus*. It is a potent naturally occurring carcinogen and has been associated with liver cancer in humans when hepatitis is present (Anderson et., 1975 and Mc Glynn et al. 1995). *Aspergillus flavus* causes significant aflatoxin accumulation in Southern U.S. growing regions (Payne, 1992) and also in the Midwestern states during years with drought stress during flowering and grain filling. As a result , the US Food and Drug Administration prohibits inter-state commerce of feed grain containing more than a maximum limit of 20 ng g^{-1} aflatoxin (Park and Liang, 1993).

Contamination of maize crop in other countries is more serious than it is in the USA, because a greater portion of this maize is directly consumed as human food. In Benin and Nigeria, maize is a primary stable food for humans and many people are exposed to aflatoxin well above accepted standards (mean 37 ppb in Benin and up to 292 ppb in Nigeria) (Cardwell et al., 1997). Evidence is overwhelming that aflatoxin contamination is present at preharvest and post harvest stages. The adverse economic effects of aflatoxin include lower yields for food and fiber crops. Farmers worldwide could loose up to 100 million dollars in form of non-marketable grain, restricted markets, increased cost of drying and selling, and yield loss (Nichols, 1983).

Several control measures such as cultural practices, insecticides, biological controls and physical detoxification have tried to reduced or eliminate aflatoxin contamination in maize, none appear to be economically feasible (Lillehoj and Wall, 1987).

Conventional Breeding

Host plant resistance has been considered as a most logical and economical way to solve the aflatoxin problem in maize (Zuber et al., 1978; Gorman and Kang, 1991; Widstrom, 1996). Several sources of resistance that exhibit reduced aflatoxin accumulation have

been identified (Campbell and White, 1995; Williams and Windham, 2001). Attempts to develop lines of corn with resistance to aflatoxin accumulation have been difficult due to the inherent problems in evaluating genotypes for resistance aflatoxin accumulation. Payne et al (1992) grouped these problems into four. The disease is greatly influenced by the environment, no good inoculation technique is available that allows selection for resistance to both the parasitic and saprophytic abilities of the fungus, the lack of a inexpensive and reliable means to evaluate resistance, and resistance must be expressed in a mature plant organ that is in a low metabolic state. Despite this, there is evidence that resistance to aflatoxin contamination is under genetic control (Zuber et al., 1978; Widstrom et al., 1984 and Thompson et al., 1984). Munkvold (2003) suggested that the best approach to reducing aflatoxin contamination is genetic resistance.

Widstrom et al. (1996) listed the following four major genetically controlled traits that could influence or condition resistance to aflatoxin contamination in the following sequence: resistance to the infection process; resistance to aflatoxin formation once infection has occurred, and resistance to insect damage and resistance to environmental stress. Testing of genotypes across years and or locations is deemed necessary because large genotype by environment interactions are often encountered in aflatoxin studies (Zhang et al., 1997). Studies have indicated that resistance to aflatoxin in maize kernels was a quantitative trait, thus making traditional breeding difficult.

Molecular Breeding

Breeding for aflatoxin is complex due to the influence of genotype by environment interaction and this also affects the genetic gain of the crops affecting by aflatoxin. Lee (1995) reported that the genetic potential of complex traits can be improved by the application of molecular markers. For progress to be achieved in molecular breeding, it is necessary to obtain information concerning the number of loci and precise location of the QTLs Stuber (1992). Identification of candidate QTL, elucidation of epistatic and pleiotropic relationships, as well as the genetic basis of heterosis may provide the

necessary tools to allow significant advances in plant improvement and elite germplasm identification (Stuber, 1992).

The ultimate goal is to develop resistant cultivars while avoiding the expensive and laborious difficult phenotyping procedures, multi environment and multiplication testing and reducing time involved by using off-season nurseries and green houses. In developing a marker assisted selection strategy, it is important to identify resistance sources and QTLs linked to the trait of the interest. The effectiveness of marker-assisted selection for quantitative trait depends on the accuracy of quantitative trait locus QTL position and effect estimates (Holland, 2004). Marker assisted selection is an effective approach for breeding resistance to several disease, one of which is aflatoxin resistance and it is necessary to understand the genetic basis of the host plant relationship. Breeding for aflatoxin resistance is different from resistance to other diseases because quantifying the amount of aflatoxin accumulation requires costly assays. Robertson et al. (2005) stated that since resistance to mycotoxin accumulation varies quantitatively among genotypes, it is expected that resistance is controlled by multiple quantitative trait loci (QTLs) and identification of DNA markers linked to resistance QTLs will allow for efficient and fast maker assisted selection program.

Quantitative Trait Loci

Resistance to aflatoxin is a complex trait that is governed by several genes and quantitative trait loci. The major goal of a QTL mapping is for use in a marker assisted selection. Once the linkage map is constructed, association between the marker alleles and the QTL might be found and utilized to develop improved lines or populations (Dudley, 1993). The acceptable markers are those that are closely linked to the desired gene or the QTL is linked to two close flanking markers. Aflatoxin has been earlier reported to have low to medium heritability (Betran et al., 2002; Betran et al., 2006) which is greatly affected by genotype by environment interaction. Detecting QTLs and accurately estimating their effects are more difficult for traits with low heritability (Beavis, 1998). The specific type of mapping population to be used depends on time

needed to complete the project, available resources, laboratory space and institutional infrastructure.

The most popular mapping population is the RIL population because can be propagated indefinitely, which allows for testing in several years and locations. Various types of markers are used for mapping and these must be polymorphic in order to distinguish between both parents. Marker types include simple sequence repeats (SSR), single nucleotide polymorphism (SNP) and restriction fragment length polymorphism (RFLP). Various types of statistical approached are used to identify association between QTL and marker locus and they include single marker analysis, interval mapping and composite marker analysis. Single marker analysis considers the association between the trait and one marker. Interval mapping considers pairs of adjacent markers as a unit and tests for the presence of a QTL within each unit by comparing flanking marker information (Lander and Boststein, 1989). Composite interval mapping (Zeng, 1994) is a combination of interval mapping and multiple regression analysis which uses specific marker loci to control for the presence of multiple QTL linked to the interval being considered. It is important to control sources of error during QTL analysis. The ability to detect the presence of a real QTL has been affected by several factors such as genotype by environment interaction and low QTL detection power. Earlier reports by Beavis (1997) and Dudley (1993) found many factors that influence the ability to detect significant associations between environment, QTL and marker loci. Such factors include gene actions of the desired trait, population size and type, environmental and experimental design.

QTLs for Aflatoxin Resistance

Aflatoxin resistance is a complex trait that is greatly influenced by the environment. Payne (1992) reported that aflatoxin accumulation is increased by hot and dry conditions. There are several prior reports on the QTL for aflatoxin resistance using different types of mapping population. Paul et al. (2003) conducted experiments to identify loci

associated with reduced aflatoxin in $F_{2:3}$ and BC1S1 generations of a susceptible line B73 and a resistant line Tex6. They detected QTLs associated aflatoxin on eight chromosomes. A total number of four QTLs associated aflatoxin was reported by Kaufman et al. (1995). Also, another study using MP313E as the resistant parent detected several QTLs in six chromosomes (Brooks et al., 2005).

Materials and Methods

Population Development

A mapping population composed of 300 recombinant inbred lines (RIL) was derived from a cross between CML161 and B73*o2*. Inbred CML161 is an exotic subtropical inbred classified as Quality Protein Maize (QPM) and released by CIMMYT. It has flint endosperm and white cobs. B73*o2* is a floury B73 carrying a non-functional mutation in the *opaque-2* gene, which regulates the expression of alpha zeins. The two parents, CML161 and B73*o2*, differ in several agronomic characteristic such as flowering dates and susceptibility to pests and diseases. The two inbreds were crossed and selfed continuously for six generations to produce the S5 RIL mapping population. A total of 146 RILs were selected out of the 300 RILs evaluated in field experiments. This selection was based on the molecular data and the 146 RILs which were almost 100% in homozygosity were selected for this mapping experiment.

Environment and Inoculation

The mapping population was evaluated in two Texas locations, Weslaco (latitude 26^{o}09, elevation 22.5 m) and College Station (latitude 30^{o}37, elevation 96m). The experimental design was an alpha lattice with three replications in Weslaco and two replications in College Station. A total of 146 maize plants were inoculated with *Aspergillus flavus* isolate NRRL3357. The method of inoculation used was the non-wounding silk channel inoculation technique (Zummo and Scott, 1989). The plants were inoculated six to ten days after midsilk using a conidial suspension containing 3 x 10^7 conidia of *A. flavus* in 3 ml of distilled water injected into the plant.

Phenotyping

Data were recorded on plot basis for all experiments on the following agronomic traits:

- Aflatoxin concentration: the whole plot grain sample was ground using a Romer mill and 50g sub samples of the flour were used to quantify aflatoxin using monoclonal antibody affinity columns and fluorescence determination using the Vicam AFLAtest (Watertown, MA). It is expressed in parts per billion (ppb or ng g^{-1}).
- Maturity: number of days from planting until the day when 50% of the plants show silks.
- Endosperm texture: visual rating of kernel in a scale 1 to 5 where 1= flinty endosperm having round crown kernel vitreous appearance and 5 = soft endosperm with marked dentations.
- Percentage of rotten ears: measured as percentage of the number of diseased ears divided by the number of inoculated ears.
- Grain yield per ear: it was estimated by dividing the total plot grain weight over the number of ears. It was reported in grams per ear.

DNA Extraction

Ten seed from 146 recombinant inbred lines were grown in a greenhouse and DNA was extracted was from the freshly emerging maize seedlings using a modified CTAB- based DNAzol protocol. Approximately 100 – 120mg of the leaf tissue was weighed and cut in bits into 1-5ml labeled microfuge tubes. Sterile metal rods were added to the tubes. The tissues were suspended in 350 ul of extraction buffer, 7ml of b-mercaptoethanol were added to each tube. 7 ul 10 mg/ml RNAase. A solution was added to the tube and the samples were ground by placing the tube on a Genogrinder for 60 seconds. The metal rods were removed using a magnetic and 350 ul of plant DNAzol was added to each tube and the mixture was inserted for 10 minutes. Exactly700 ul of the supernatant was transferred to new tubes and equal volume of chloroform was added and centrifuged for 10 min at 130^0 rpm. DNA was precipitated with 0.75 volume of 100% ethanol and then pelleted by centrifugation at 13000 rpm. The DNA pellets were washed by discarding the supernatant and addition of 150 ul of DNAzol ethanol wash solution for 5 minutes and 500ul 75% ethanol twice. The DNA pellets were centrifuged for 30 minutes to collect the wash pellet by discarding the supernatant and air dried for two to three hours. The DNA pellet was dissolved by addition of 35ul ½ x TE finger tapped and placed in 45^0 water bath for 5 minutes. The DNA samples were quantified using fluorometer TD – 360 (Turner Design Inc.) and diluted using 1 x TE buffer to a concentration of 10ng ul^{-1}.

Marker Amplification and Linkage Map Construction

The markers used in this experiment were simple sequence repeat (SSR) markers (Guo et al., 2001). They were selected because they are highly polymorphic, repeatable, and are based on polymerase chain reaction (PCR). Maize SSR markers were selected from Maize Genetics and Genomics Database (www.maizedgb.org) to referenced loci location**.**

The markers were first screened on the parental lines to test for polymorphism and then, polymorphic markers were run on the entire population. A total 350 SSR marker primer pairs were screened and only 143 were polymorphic.

The PCR reaction mixture consists of 1.9ul of water, 1.0ul of 10x Buffer Promega, 1.0UL of 25mM $MgCL_2$, 0.6ul of dNTPs (2.5mM) 1.0ul of 100% glycerol, 1ul of 2.5 ul of forward and reverse primer, 0.1ul of Taq polymerase (5U/ul) and 2.0 UL (20ng) of DNA. The amplification conditions were one cycle of 93^0 C for one minute, thirty five cycles of 93^0C for 30 seconds, annealing for 1 min (55 - 66 ^{0}C) followed by 72^0 C for 1 min and followed by 72 0C for 5 minutes. It is summarized as the Denaturing, Annealing and Elongation steps. PCR reactions were run on B1 Gene Amp 2700 or 9700 thermocyclers.

Amplification products were visualized and electrophoresized in a 4 % Superfine Resolution Agarose SFR gel stained with ethidium bromide. The gels were run for 2-3 hrs at 300 volts and then exposed to ultraviolet light. The mapping gel was scored as A for the fragment generated by the B73*o2* parent and B for the fragment generated by the CML 161 parents. The heterozygotes were considered missing data.
Recombination frequencies and map distances were estimated using mapmaker/ E&P 3.0 (whitehead Institute, Cambridge, MA). Kosambi mapping function was used to transform the recombination frequencies into CentiMorgans (cM).

Statistical Analysis

Data was collected on aflatoxin and four secondary traits, maturity, percentage of rotten ears, endosperm texture and grain yield. Aflatoxin concentration in ppb was transformed to logarithm to normalize the variance. Analysis for individual environments were carried on using REMLtool (Welen, 2003) as alpha-lattice with and without spatial analysis and randomized complete block design and adjusted means were obtained as those with the lowest mean square error. Across environments analysis was conducted

using PROC GLM in SAS 9.0 (SAS Institute, 2002). The genotypes, replications and environment were considered random.

Single marker analysis was conducted for all the traits using the phenotypic data and marker data to test the significance of each marker for the trait in question. Association between the marker locus and the trait were detected with a significance of $P \leq 0.05$. Composite interval mapping (CIM) was conducted using QTL cartographer version 2.5 (Zeng, 1993 and 1994). The forward and backward regression method was selected to perform the composite interval mapping. The threshold LOD score to declare 0.05 significance threshold for QTL was estimated after 1000 permutations (Doerge and Churchill, 1996; Doerge and Rebai, 1996). QTL analysis was performed for each trait within each environment and across all environments. All traits were tested for digenic epistatic interaction using SAS EPISTACY program (Holland, 1998). Pair wise interactions of $P \leq 0.001$ were considered significant. Partial R^2 parameter was determined for the individual interaction effect. Holland et al. (1997) defined partial R^2 as the amount of phenotypic variation explained by the interaction effect after accounting for the main effects of loci. This was calculated by dividing the type III sums of squares for the model which included both main effects plus the interaction effects by the total sums of squares.

Results

Aflatoxin

In the single marker analysis, seven and five markers were significantly associated with aflatoxin infection in Weslaco and College Station (Table 4.1) In nine of the markers, the CML161 allele was associated with lower aflatoxin concentration while for the remaining three markers, the B73*o2* allele was associated with lower aflatoxin. Results from likelihood ratio tests statistics (LR) generated by the permutation test in CIM, only one QTL in chromosome 1 was detected in Weslaco, none in College Station and two in chromosome 1 across both locations (Table 4.2). The QTL in chromosome 1 detected in Weslaco accounts for 19% of the phenotypic variation while the two QTLs detected across environments accounted for 36% of the phenotypic variation. One of the QTLs is located near bnlg1953 and is associated with 21% of the phenotypic variation. The second QTL is located between umc2096 and umc1917 and accounts for 15% of the phenotypic variation. Six putative QTLs were detected by lowering the permutation LOD to 2.5 in Weslaco and College Station. These QTLs were detected in chromosomes 1, 3, 6, 7 and 9 accounting for very small percentage of phenotypic variation (Table 4.2, Figure 4.1). Two QTLs were detected in chromosome 1 for the across environments analysis and they were flanked by markers bnlg 1953 – bnlg 2204 and umc 2096 – umc 1917.

Overall the environments, three QTLs were identified for aflatoxin resistance with the allele that reduces aflatoxin derived from the CML161 parent (Table 4.2). The total phenotypic variation for Weslaco, College Station and across locations explained by markers was 41%, 49% and 40%, respectively. The aflatoxin resistance QTL with the largest effect was on chromosome 1 and is located between bnlg 1953 and bnlg 2204 and explains 21% of the phenotypic variation.

Epistasis refers to allelic interaction at several loci and this can play an important role between QTLs. The markers were evaluated pair wise for significant epistatic interaction using LS means. For aflatoxin (ppb), thirty-eight digenic epistatic interactions were significant (Table 4.3) at P>0.001. In Weslaco, the interaction with the highest partial R^2 value was the interaction between unc1136 and umc1257 accounting for 12% of the total phenotypic variation in Weslaco, 21% in College Station, and 10% in across locations, after accounting for main effect of the markers .

Epistasis effects were not consistent in each location and combined location analysis. Only 5 of the 38 digenic epistatic interactions involved loci which had one locus with a significant main effect. The remaining 33 interaction involved loci which both had no significant main effect.

Table 4.1. Markers in single marker analysis significantly associated with aflatoxin concentration (ng g^{-1}) in CML161xB73*o2* recombinant inbred line population in Weslaco and College Station, TX in 2005.

Location	Position	Marker	R^2	B73	CML	Additive Effect
Weslaco	1.04	Umc 1917**	0.058	1270	941	-164.6**
Weslaco	1.03	Umc 2096**	0.154	1399	845	-276.6**
Weslaco	1.03	BNLG 2204**	0.11	1375	913	-231.2**
Weslaco	6.05	Umc 1805**	0.1	1379	919.2	-230.22**
Weslaco	6.06	Umc 1912*	0.02	1255	1014.4	-120.65*
Weslaco	9.01	Umc 2393*	0.04	1262	969.8	-146.19*
Weslaco	9.01	bnlg 1724**	0.08	1355	954.5	-200.26**
College Station	1.04	Umc 1917**	0.06	2173	1451.1	-360.94*
College Station	1.04	Umc 2112*	0.04	2140.9	1529	-305.92*
College Station	3.07	Txp 196L**	0.12	1151.8	2168	508.13**
College Station	3.08	Txp 218 L	0.05	1440.46	2104	332.11*
College Station	7.02	Umc 2142**	0.115	1235.96	2298	531.21**

* Significant at the 0.05 level of

** Significant at the 0.01 level

Table 4.2. Chromosome and map positions, nearest flanking marker loci, effects and variance associated with QTL identified with CIM in CML 161xB73*o2* recombinant inbred line population in Weslaco, College Station and across locations for aflatoxin concentration (ng g^{-1}).

Env	Chr	Pos	Lmarker	Bin	Rmarker	Bin	Lod	R^2	Add	FAllele
WE	1	81.7	umc2096	1.03	umc1917	1.04	6.404	19	310	CML 161
	6	**89.7**	**umc1805**	**6.05**	**umc1912**	**6.06**	**2.738**	**7**	**193**	**CML 161**
	9	**3.2**	**bnlg1724**	**9.01**	**umc2393**	**9.01**	**1.410**	**6**	**177**	**CML 161**
CS	**1**	**98.0**	**umc1917**	**1.04**	**umc2112**	**1.05**	**1.523**	**6**	**366**	**CML 161**
	3	**136.7**	**umc1644**	**3.06**	**txp218L**	**3.08**	**2.320**	**8**	**-416**	**B 73o2**
	7	**81.1**	**umc2142**	**7.02**	**mmc0411**	**7.02**	**1.641**	**7**	**-379**	**B 73o2**
	9	**21.4**	**umc2393**	**9.01**	**umc2093**	**9.01**	**1.491**	**9**	**436**	**CML 161**
Across	1	69.0	bnlg 1953	1.02	bnlg2204	1.03	5.318	21	403	CML 161
	1	83.5	umc2096	1.03	umc1917	1.04	4.620	15	343	CML 161

Chr =chromosome, R-marker=Right marker,L-Marker =Left marker.
FAllele=Favorable Allele, Add =Additive effect, Pos=Position
Bold lettering indicates putative QTL according to lowered permutation LOD
R^2 indicates percentage of phenotypic variation explained (PVE)by the locus.

Table 4.3. Significant epistatic interactions between loci in CML 161xB73*o2* recombinant inbred line population in Weslaco, College Station and across locations for aflatoxin concentration (ng g^{-1}).

Location	locus1	locus2	probint	partR2
Weslaco	BNLG1019	UMC2258	0.0005	0.0956
	BNLG1137	UMC1345	0.0006	0.0800
	BNLG1137	UMC2180	0.0004	0.0867
	BNLG2191	UMC1136	0.0010	0.0889
	BNLG2291	UMC1345	0.0008	0.0800
	BNLG2291	UMC2180	0.0009	0.0812
	NC003	UMC1101	0.0003	0.0984
	PHI064	UMC2246	0.0004	0.0897
	PHI085	UMC1644	0.0008	0.0850
	PHI087	UMC1644	0.0010	0.0847
	UMC1136	UMC1257	0.0001	0.1223
	UMC1143	UMC1639	0.0005	0.1004
	UMC2012	UMC2180	0.0001	0.1034
College Station	TXP100L	UMC2152	0.0003	0.1525
	BNLG118	BNLG2204	0.0000	0.1912
	BNLG2248	UMC2128	0.0003	0.2163
	BNLG244	UMC1122	0.0009	0.1354
	PHI114	UMC2152	0.0002	0.1697
	PHI127	UMC2281	0.0001	0.1890
	UMC1161	UMC1804	0.0009	0.1398
	UMC1553	UMC2152	0.0007	0.1410
	UMC1710	UMC2152	0.0006	0.1453
Across	TXP46L	UMC1147	0.0001	0.1083
	BNLG1137	UMC2180	0.0003	0.0919
	BNLG1154	UMC1460	0.0003	0.0932
	BNLG1953	PHI041	0.0010	0.0821
	BNLG244	UMC1551	0.0008	0.0799
	PHI046	UMC1127	0.0008	0.0760
	PHI085	UMC2170	0.0003	0.0984
	PHI087	UMC2170	0.0007	0.0904
	PHI127	UMC2281	0.0002	0.0949
	UMC1041	UMC1652	0.0007	0.0861
	UMC1041	UMC2142	0.0009	0.0822
	UMC1042	UMC1161	0.0004	0.1082
	UMC1553	UMC2152	0.0009	0.0821
	UMC1562	UMC1970	0.0003	0.0897
	UMC1562	UMC2071	0.0006	0.0834
	UMC1775	UMC2180	0.0005	0.0924

Probint = Probability value for interaction. PartR2 =Partial R2 value

Logarithmic Aflatoxin

In single marker analysis, five and four markers were significantly associated with aflatoxin reduction in Weslaco and College Station, (Table 4.4).. In Weslaco, all five markers associated lower aflatoxin concentration with the CML161 allele while in College Station three out of the four markers associated the B73*o2* allele with lower aflatoxin concentration.

Results from likelihood ratio test statistics (LR) generated by the permutation test in CIM, three QTLs were detected in Weslaco, one QTL in College Station and three QTLs across both environments (Table 4.5, Figure 4.1). The QTLs detected in Weslaco were located in chromosomes 1, 6 and 9 and they account for 12%, 11% and 9%, respectively with the allele that contributes to aflatoxin reduction derived from the CML161 parent.

In College Station, only one QTL was detected in chromosome 3 accounting for 14% of the phenotypic variation. In the across location analysis, three QTLs were detected in chromosomes 1, 3 and 5 accounting for 13%, 6% and 9%, respectively. In College Station three putative QTLs were detected in chromosomes 1 and 7 when the permutations LOD were lowered to 2.5. The CML161 parent accounts for the lower aflatoxin accumulation in chromosome 1 while the B73*o2* parent is responsible for chromosomes 3 and 5.

Across all environments, seven QTLs were identified for logarithmic aflatoxin concentration and the alleles that contribute to reduced aflatoxin accumulation were contributed from both parents. The total phenotypic variation explained by markers for Weslaco, College Station and across location was 38%, 55% and 38%, respectively. The QTL with the largest effect was on chromosome 3 and it explains 14% of the total phenotypic variation.

In this study thirty-five digenic epistatic interactions (Table 4.6) were significant at $P \leq 0.001$. The highest partial R^2 value for Weslaco, College Station and across locations was 12%, 40% and 10%, respectively after accounting for main effects of the loci.

Only 7 of the 35 digenic epistatic interactions involved loci which had one locus with a significant main effect. The remaining 28 interaction involved loci which both had no significant main effect.

Table 4.4. Markers single marker analysis significantly associated with logarithmic aflatoxin concentration in CML161xB73*o2* recombinant inbred line population in Weslaco and College Station, TX in 2005.

Location	Position	Marker	R^2	B73	CML	Additive Effect
Weslaco	1.04	Umc 1917*	0.05	2.92	2.77	-0.07
Weslaco	1.03	Umc 2096**	0.153	2.97	2.71	-0.13
Weslaco	1.03	bnlg 2204**	0.121	2 .97	2.74	-0.11**
Weslaco	6.04	Umc 1014*	0.04	2.94	2.79	-0.07*
Weslaco	6.05	bnlg 1154**	0.05	2.96	2.79	-0.08**
College Station	1.06	Umc 1590**	0.13	3.16	2.86	-0.15**
College Station	3.06	UMC 1644*	0.1	2.85	3.13	0.13*
College Station	3.07	Txp 196L**	0.18	2.77	3.15	0.19**
College Station	3.08	Txp 218L	0.07	2.9	3.12	0.11

* Significant at the 0.05 level of
** Significant at the 0.01 level

Table 4.5. Chromosome and map positions, nearest flanking marker loci, effects and variance associated with QTL identified in CML161xB73*o2* recombinant inbred line population in Weslaco, College Station and across locations for logarithmic aflatoxin concentration.

Env	Chr	Pos	Lmarker	Bin	Rmarker	Bin	Lod	R^2	Add	FAllele
WE	1	79.4	umc2096	1.03	umc1917	1.04	3.648	12	0.12	CML 161
	6	60.0	umc1014	6.04	bnlg1154	6.05	3.207	8	0.11	CML 161
	9	3.0	bnlg1724	9.01	umc2393	9.01	3.541	8	0.09	CML 161
CS	3	136.8	umc1644	3.06	txp169 L	3.07	3.69	14	-0.16	B 73o2
	1	**32.1**	**umc2012**	**1.01**	**bnlg1429**	**1.02**	**1.667**	**11**	**0.15**	**CML 161**
	1	**125.0**	**umc1703**	**1.05**	**umc1590**	**1.06**	**1.499**	**5**	**0.11**	**CML 161**
	7	**158.6**	**umc1710**	**7.04**	**umc1782**	**7.04**	**1.137**	**4**	**-0.09**	**B 73o2**
Across	1	76.2	bnlg2204	1.03	umc2096	1.06	4.557	13	0.14	CML 161
	3	145.5	umc2274	3.08	phi046	3.08	2.693	6	-0.14	B 73o2
	5	236.5	bnlg118	5.07	bnlg386	5.09	2.359	9	-0.11	B 73o2

Chr =chromosome, R-marker=Right marker,L-Marker =Left marker.
FAllele=Favorable Allele, Add =Additive effect, Pos=Position
Bold lettering indicates putative QTL according to lowered permutation LOD
R^2 indicates percentage of phenotypic variation explained (PVE)by the locus.

Table 4.6. Significant epistatic interactions between loci in CML161xB73*o2* recombinant inbred line population in Weslaco, College Station and across locations for logarithmic aflatoxin concentration.

Location	locus1	locus2	probint	partr2
Weslaco	BNLG1019	UMC2258	0.0007	0.0891
	BNLG1208	UMC1327	0.0007	0.0882
	BNLG1666	BNLG1671	0.0007	0.0793
	BNLG1671	UMC1534	0.0004	0.0838
	NC003	UMC1101	0.0009	0.0823
	PHI064	UMC2246	0.0003	0.0936
	PHI087	UMC1644	0.0005	0.0933
	UMC1041	UMC2180	0.0007	0.0855
	UMC1143	UMC1639	0.0003	0.1041
	UMC1460	UMC2011	0.0002	0.0946
	UMC2012	UMC2180	0.0001	0.1134
College Station	BNLG1523	UMC2111	0.0008	0.2951
	BNLG1714	UMC1456	0.0000	0.4059
	BNLG1724	UMC1101	0.0005	0.2811
	BNLG1724	UMC2011	0.0001	0.3303
	UMC1101	UMC2393	0.0006	0.2853
	UMC1760	UMC2018	0.0004	0.3048
	UMC1957	UMC2011	0.0000	0.3829
	UMC2011	UMC2393	0.0001	0.3367
Across	TXP100L	UMC1456	0.0008	0.0766
	TXP100L	UMC2152	0.0002	0.0959
	TXP46L	UMC1147	0.0009	0.0820
	BNLG1019	UMC2258	0.0008	0.0891
	BNLG1154	UMC1460	0.0008	0.0790
	BNLG1208	UMC1327	0.0010	0.0846
	BNLG1429	UMC1912	0.0004	0.0938
	BNLG244	UMC1551	0.0002	0.0996
	PHI064	UMC1426	0.0008	0.0809
	PHI114	UMC1015	0.0009	0.0768
	PHI114	UMC1456	0.0005	0.0849
	PHI114	UMC2098	0.0009	0.0782
	PHI114	UMC2152	0.0002	0.0972
	UMC1042	UMC1161	0.0004	0.1072
	UMC1042	UMC1460	0.0009	0.0887
	UMC1562	UMC1970	0.0005	0.0849

Probint = Probability value for interaction Part R^2 = Partial R^2 value

Maturity

In single marker analysis, three and five markers were significantly associated with female flowering in Weslaco and College Station, (Table 4.7). Results from the composite interval marker analysis (Table 4.8, Figure 4.1) indicate that one QTL was detected in College Station, none in Weslaco and five QTLs across locations. The first QTL College Station was located in chromosome 2 between markers umc2030 and umc1635 and accounts for 4% of the total phenotypic generation. The second QTL was located in chromosome 8 between umc1034 and bnlg1006 and accounts for 6% of the total phenotypic variation. In Weslaco, no QTL were detected, however, when the permutation LOD were lowered to 2.0, four QTLs were detected in chromosomes 1, 2, 5, and 7. Across locations five QTLs in chromosomes 5, 6, 8, 8 and 9 account for 3%, 2% ,7%, 5% and 4%, respectively, of the total phenotypic variation. All QTLs were contributed from both parents. In Weslaco, the total phenotypic variation accounts for 19%, 24% in College Station and 20% for across locations.

In this study, twenty-two digenic epistatic interaction were significant at P>0.001 (Table 4.9). In Weslaco, the interaction with the highest partial R^2 was between umc1797 and umc2142 accounting for 9% of the total phenotypic variation. 11% in College Station, and 9% in across both location after accounting for main effects of the loci.

Only 5 of the 22 digenic epistatic interactions involved loci which had one locus with a significant main effect. One of the interactions involved loci which both had significant main effect. The remaining 16 interaction involved loci which both had no significant main effect.

Table 4.7. Marker single marker analysis significantly associated with maturity in CML161xB73*o2* recombinant inbred line population in Weslaco and College Station.

Location	Position	Marker	R^2	B73	CML	Additive Effect
Weslaco	1.07	Umc 1147*	0.02	64.3	66.05	0.87*
	2.01	Umc 2094*	0	64.16	65.9	0.86*
	7.02	Umc 1016*	0.02	64.66	65.66	0.49*
College Station	2.07	Umc 2030**	0.06	70.9	69.92	-0.5*
	2.05	Umc 1635	0.03	70.8	70.04	-0.37*
	6.05	Umc 1805	0.02	70.63	70.04	-0.29*
	6.04	Phi 45269	0.01	70.6	70.08	-0.26*
	8.02	Umc 1304	0.05	69.84	70.71	0.43*

* Significant at the 0.05 level of
** Significant at the 0.01 level

Table 4.8. Chromosome and map positions, nearest flanking marker loci, effects and variance associated with QTL identified in CML 161xB73*o2* recombinant inbred line population in Weslaco, College Station and across locations for maturity.

Env	Chr	Pos	Lmarker	Bin	Rmarker	Bin	Lod	R2	Add	FAllele
WE	**1**	**167.5**	**umc1833**	**1.07**	**umc1147**	**1.07**	**1.52**	**6**	**-1.43**	**B 73o2**
	2	**10.4**	**umc2094**	**2.01**	**umc1165**	**2.01**	**1.65**	**5**	**-1.28**	**B 73o2**
	5	**4.7**	**bnlg1006**	**5**	**phi024**	**5.01**	**1.12**	**3**	**1.08**	**CML 161**
	7	**59.6**	**umc1016**	**7.02**	**phi034**	**7.02**	**1.06**	**3**	**-1.06**	**B 73o2**
CS	2	83.9	umc2030	2.04	umc1635	2.05	1.26	4	0.4	CML 161
	6	**82.3**	**phi452693**	**6.04**	**umc1805**	**6.05**	**1.26**	**4**	**0.4**	**CML 161**
	8	**25.9**	**umc1034**	**8.02**	**bnlg1006**	**5**	**1.76**	**6**	**-0.47**	**B 73o2**
Across	5	11.3	bnlg1006	5	phi024	5.01	1.27	3	1.2	CML 161
	6	73.5	phi452693	6.04	umc1805	6.05	1.07	2	1.07	CML 161
	8	22	phi42070	8	umc1304	8.02	2.50	7	-1.96	B 73o2
	8	43.2	phi10017	8.04	umc1460	8.04	2.00	5	1.72	CML 161
	9	87.7	umc2128	9.03	phi065	9.03	1.45	4	-1.31	B 73o2

Chr =chromosome, R-marker=Right marker,L-Marker =Left marker.
FAllele=Favorable Allele, Add =Additive effect, Pos=Position
Bold lettering indicates putative QTL according to lowered permutation LOD
R^2 indicates percentage of phenotypic variation explained (PVE)by the locus.

Table 4.9. Significant epistatic interactions between loci in CML 161xB73*o2* recombinant inbred line population in Weslaco, College Station and across locations for maturity.

Location	locus1	locus2	probint	partr2
Weslaco	BNLG1714	UMC2214	0.0008	0.0854
	UMC1122	UMC1805	0.0009	0.0910
	UMC1147	UMC2094	0.0005	0.0863
	UMC1147	UMC2246	0.0010	0.0824
	UMC1506	UMC2281	0.0005	0.0926
	UMC1797	UMC2142	0.0007	0.0958
	UMC2039	UMC2093	0.0003	0.0952
	UMC2093	UMC2281	0.0003	0.0901
College Station	BNLG1006	UMC1551	0.0007	0.0878
	BNLG1046	UMC1590	0.0009	0.0897
	PHI024	UMC1545	0.0001	0.1105
	UMC1041	UMC1703	0.0005	0.0910
	UMC1041	UMC1917	0.0001	0.1121
	UMC1041	UMC2112	0.0005	0.0949
	UMC1703	UMC2012	0.0003	0.0909
	UMC1917	UMC2012	0.0001	0.1091
	UMC2012	UMC2025	0.0001	0.1038
	UMC2012	UMC2112	0.0004	0.0941
Across	BNLG2248	UMC1587	0.0006	0.0965
	PHI085	UMC1590	0.0005	0.0930
	UMC1165	UMC2170	0.0007	0.0955
	UMC1551	UMC2018	0.0009	0.0832

Probint = Probability value for interaction. Part R^2 = Partial R^2 value

Percentage of Rotten Ears

In single marker analysis,six and three markers were significantly associated with percentage of rotten ears in both College Station and Weslaco (Table 4.10) The CML161 allele was associated with lower percentage of rotten ears. Marker umc1307 had the highest additive effect (-8.86) and accounted for the highest phenotypic variation of 8%. In Weslaco one QTL was identified in chromosome 2 between markers umc1024 and umc2030 accounting for 19% of the total phenotypic variation. In College Station, no QTL was detected, but when the permutation LOD was lowered to 2.5, one putative QTL was detected in chromosome 3. Across both locations, only one QTL was in found in chromosome 3 between markers umc1307 and umc1538 and it accounts for 28% of the total phenotypic variation (Table 4.11, Figure 4.1). Low percentage of ear rot at all three QTLs detected was contributed from the CML161 allele.

In this trait, a total number of thirty one digenic epistatic interaction were significant at P >0.001 (Table 4.12). In Weslaco, the interaction with the highest partial R^2 value was the interaction between txp00L and phi10017, phi10104, phi10222 and phi10841 and this five interactions all account for 10% of the total phenotypic variation. The interaction between umc1976 and umc2012 accounts for the highest partial R^2 of 10%.in College Station. In across locations the interaction between bnlg1523 and umc1058 had the highest partial R^2 of 9%. Only 3 of the 31 digenic epistatic interactions involved loci which had one locus with a significant main effect. The remaining 28 interaction involved loci which both had no significant main effect.

Table 4.10. Markers single marker analysis significantly associated with percentage of rotten ears in CML161xB73*o2* recombinant inbred line population in Weslaco and College Station.

Location	Position	Marker	R^2	B73	CML	Additive Effects
Weslaco	2.05	Umc 1635*	0.03	23.24	16.1	-3.56*
Weslaco	2.04	Umc 1024**	0.07	24.77	13.97	-5.39**
Weslaco	2.03	Umc 1555**	0.06	23.97	14.39	-4.78**
Weslaco	3.05	Umc 1307**	0.06	25.77	15.38	-5.19
Weslaco	3.05	Umc 1539*	0.02	22.6	16.22	-3.18
Weslaco	3.04	Bnlg 1019*	0.02	47.23	39.18	-4.02
College Station	3.05	Umc 1307**	0.08	54.78	37.05	- 8.86**
College Station	3.05	Umc 1539**	0.02	46.28	38.01	-4.13*
College Station	3.09	Umc 2152**	0.07	49.93	35.35	-7.29**

* Significant at the 0.05 level

** Significant at the 0.01 level

Table 4.11. Chromosome and map positions, nearest flanking marker loci, effects and variance associated with QTL identified in CML 161xB73*o2* recombinant inbred line population in Weslaco, College Station and across locations for percentage of rotten ears.

Env	Chr	Pos	Lmarker	Bin	Rmarker	Bin	Lod	R^2	Add	FAllele
WE	2	71.4	umc1024	2.04	umc2030	2.04	5.21	19	8.85	CML 161
CS	***3**	**186.9**	**umc2152**	**3.09**	**umc1639**	**3.09**	**2.85**	**17**	**1078**	**CML 161**
Across	3	81.9	umc1307	3.05	umc1539	3.05	4.90	28	10.31	CML 161

Chr =chromosome, R-marker=Right marker,L-Marker =Left marker.

FAllele=Favorable Allele, Add =Additive effect, Pos=Position

Bold lettering indicates putative QTL according to lowered permutation LOD

R^2 indicates percentage of phenotypic variation explained (PVE)by the locus.

Table 4.12. Significant epistatic interactions between loci in CML161xB73*o2* recombinant inbred line population in Weslaco, College Station and across locations for percentage of rotten ears.

Location	locus1	locus2	probint	partr2
Weslaco	TXP100L	PHI087	0.0001	0.1187
	TXP100L	PHI10017	0.0001	0.1187
	TXP100L	PHI10104	0.0001	0.1187
	TXP100L	PHI10222	0.0001	0.1187
	TXP100L	PHI10841	0.0001	0.1187
	BNLG1523	UMC1058	0.0007	0.0860
	BNLG244	UMC1551	0.0001	0.1091
	PHI087	PHI114	0.0001	0.1135
	PHI087	UMC1710	0.0001	0.1141
	UMC1545	UMC1966	0.0003	0.0928
	UMC1553	UMC2096	0.0004	0.0966
	UMC1587	UMC2115	0.0002	0.1057
	UMC2093	UMC2258	0.0002	0.0947
College Station	BNLG1523	UMC2152	0.0009	0.0730
	UMC1058	UMC2098	0.0008	0.0809
	UMC1307	UMC1551	0.0009	0.0755
	UMC1327	UMC1456	0.0007	0.0827
	UMC1327	UMC1805	0.0007	0.0910
	UMC1456	UMC2152	0.0003	0.0835
	UMC1833	UMC2258	0.0001	0.1077
	UMC1976	UMC2012	0.0003	0.0942
Across	BNLG1523	UMC1058	0.0002	0.0980
	BNLG1523	UMC1180	0.0008	0.0820
	BNLG1523	UMC2011	0.0007	0.0809
	BNLG244	UMC1551	0.0002	0.1005
	PHI034	UMC1663	0.0008	0.0864
	PHI087	UMC1710	0.0008	0.0903
	UMC1058	UMC1307	0.0008	0.0759
	UMC1663	UMC2115	0.0009	0.0831
	UMC1746	UMC2011	0.0009	0.0812
	UMC2111	UMC2149	0.0010	0.0783

Probint = Probability value for interaction. Part R^2 = Partial R^2 value

Endosperm Texture

Single marker analysis detected seven and nine markers that were significantly associated with endosperm texture in Weslaco and College Station (Table 4.13). In nine of the markers, the CML allele bearing a negative regression coefficient was associated with a lower endosperm rating corresponding to a flinty texture. Significant differences were detected in the number of QTLs observed and percentages of phenotypic variation. In Weslaco, four QTLs were detected in chromosomes 3, 3, 6, and 8 accounting for 11%, 6%, 10% and 15% of the phenotypic variation, respectively. Two QTLs were found in chromosome 3 (Table 4.14, Figure 4.1). In College Station, two QTLs were located in chromosomes 3 and 10 and they account for 7% and 3%, respectively. While in the across location analysis five QTLs were detected in chromosomes 3, 3, 4, 6 and 8 accounting for 28%, 24%, 12%, 14% and 31%, respectively, of the total phenotypic variation. Over all the three environments, eleven QTLs were identified but the QTL with the largest effect were found in chromosome 8 explaining 15% and 17% of the total phenotypic variation. The total phenotypic variation in Weslaco, College Station and across locations was 59%, 48% and 65%, respectively. Both parental alleles CML161 and B73*o2* contributed to the QTLs detected for this trait.

Forty-six digenic epistatic interactions were significant at P>0.001 (Table 4.15). In Weslaco, bnlg2248 and umc2190 had highest partial R^2 value of 12% 13% in College Station and 11% in across location after accounting for the main effects of the loci. Only 11 of the 46 digenic epistatic interactions involved loci which had one locus with a significant main effect. The remaining 35 interaction involved loci which both had no significant main effect.

Table 4.13. Markers single marker analysis significantly associated with endosperm texture in CML161xB73*o2* recombinant inbred line population in Weslaco and College Station.

Location	Position	Marker	R^2	B73	CML	Additive Effect
Weslaco	3.03	Umc 2258**	0.05	3.53	3.14	-0.19**
	3.06	Umc 1644**	0.2	3.7	3.01	-0.34**
	3.07	Txp 196 L**	0.21	3.72	2.98	-0.37**
	3.08	TXP 218 L**	0.15	3.66	3.03	-0.31**
	6.06	Umc 2170**	0.11	2.96	3.48	0.26**
	6.07	UMC 2165**	0.08	3.01	3.46	0.22**
	8.05	Umc 1562**	0.27	3.77	2.96	-0.40**
College Station	3.04	bnlg 1019**	0.14	3.59	2.96	-0.32**
	9.02	Umc 1037**	0.12	3.68	3.03	-0.33**
	10.05	Umc 1506**	0.05	3.59	2.96	-0.18**
	10.06	Umc 1061**	0.09	3.44	2.95	-0.24**
	1.06	Umc 1590*	0.04	2.99	3.34	0.17
	1.07	Umc 1122**	0.09	2.94	3.43	0.24
	1.09	Umc 2047*	0.04	3.35	3.02	-0.16*
	6.06	Umc 2170**	0.09	2.91	3.39	0.24**
	6.06	Umc 2165**	0.06	2.99	3.39	0.19**

* Significant at the 0.05 level of
** Significant at the 0.01 level

Table 4.14. Chromosome and map positions, nearest flanking marker loci, effects and variance associated with QTL identified in CML161xB73*o2* recombinant inbred line population in Weslaco, College Station and across locations for endosperm texture.

Env	Chr	Pos	Lmarker	Bin	Rmarker	Bin	Lod	R^2	Add	FAllele
Weslaco	3	52.3	umc2258	3.03	phi37411	3.02	5.575	11	0.27	CML 161
	3	136.8	phi102228	3.06	umc1644	3.06	3.750	6	0.21	CML 161
	6	118.3	umc2170	6.06	umc2165	6.07	4.303	10	-0.25	B 73o2
	8	74.7	umc1460	8.03	umc1562	8.05	6.304	15	0.32	CML 161
College Station	3	63.7	bnlg1019	3.04	umc1307	3.05	2.999	7	3.44	CML 161
	10	87	umc1061	10.1	umc1556	10.1	0.690	3	2.12	CML 161
	1	**139.5**	**umc1590**	**1.06**	**umc1122**	**1.06**	**2.864**	**9**	**-0.24**	**B 73o2**
	1	**179.9**	**umc1147**	**1.07**	**umc2047**	**1.09**	**2.613**	**7**	**0.23**	**CML 161**
	6	**114.6**	**umc2170**	**6.06**	**umc2165**	**6.07**	**2.591**	**7**	**-0.22**	**B 73o2**
Across	3	52.3	umc2258	3.03	phi37411	3.02	0.028	13	0.28	CML 161
	3	134.3	phi102228	3.06	umc1644	3.06	0.020	9	0.24	CML 161
	4	151.3	umc1775	4.08	umc1101	4.09	1.690	2	0.12	CML 161
	6	114.6	umc2170	6.06	umc2165	6.07	1.567	3	0.13	CML 161
	8	72.5	umc1460	8.03	umc1562	8.05	6.380	17	0.31	CML 161

Chr =chromosome, R-marker=Right marker,L-Marker =Left marker.
FAllele=Favorable Allele, Add =Additive effect, Pos=Position
Bold lettering indicates putative QTL according to lowered permutation LOD
R^2 indicates percentage of phenotypic variation explained (PVE)by the locus.

Table 4.15. Significant epistatic interactions between loci in CML161xB73*o2* recombinant inbred line population in Weslaco, College Station and across locations for endosperm texture.

Location	locus1	locus2	probint	partr2
Weslaco	BNLG1046	UMC2039	0.0006	0.0925
	BNLG1046	UMC2281	0.0004	0.0979
	BNLG1137	UMC1456	0.0003	0.0886
	BNLG2248	UMC2190	0.0001	0.1252
	BNLG2291	UMC1456	0.0001	0.1137
	PHI024	UMC2246	0.0008	0.0853
	UMC1058	UMC2094	0.0002	0.0930
	UMC1136	UMC1590	0.0007	0.0887
	UMC1136	UMC1976	0.0008	0.0863
	UMC1147	UMC1506	0.0003	0.1042
	UMC1180	UMC2094	0.0001	0.1019
	UMC1231	UMC1966	0.0006	0.0816
	UMC1456	UMC2027	0.0003	0.0913
	UMC2096	UMC2184	0.0002	0.1051
College Station	BNLG1006	BNLG1208	0.0000	0.1385
	BNLG1006	UMC2111	0.0001	0.1144
	BNLG1047	UMC1304	0.0005	0.0849
	BNLG1523	UMC1180	0.0003	0.0869
	BNLG1953	UMC1143	0.0004	0.1087
	PHI065	UMC1101	0.0007	0.0861
	PHI079	UMC1101	0.0001	0.1090
	UMC1101	UMC1492	0.0002	0.1040
	UMC1143	UMC1917	0.0003	0.1023
	UMC1231	UMC1966	0.0002	0.0961
	UMC1833	UMC2258	0.0007	0.0836
Across	BNLG1019	UMC1551	0.0007	0.0751
	BNLG1137	UMC1456	0.0009	0.0760
	BNLG1523	UMC1058	0.0006	0.0840
	BNLG1523	UMC1180	0.0004	0.0836
	BNLG1755	UMC1101	0.0008	0.0731
	BNLG2248	UMC2190	0.0005	0.0966
	BNLG2291	UMC1456	0.0000	0.1210
	BNLG386	UMC1231	0.0009	0.0780
	BNLG391	UMC1327	0.0008	0.0910
	PHI024	UMC2246	0.0007	0.0874
	PHI079	UMC1101	0.0003	0.0873
	UMC1042	UMC1804	0.0009	0.0862
	UMC1058	UMC2094	0.0001	0.1048
	UMC1101	UMC1652	0.0005	0.0798
	UMC1147	UMC1506	0.0009	0.0878
	UMC1180	UMC2094	0.0001	0.1035
	UMC1231	UMC1966	0.0000	0.1128

Table 4.15 Continued. Significant epistatic interactions between loci in CML161xB73*o2* recombinant inbred line population in Weslaco, College Station and across locations for endosperm texture.

Location	locus1	locus2	probint	partr2
	UMC1456	UMC2027	0.0003	0.0918
	UMC1703	UMC2142	0.0008	0.0815
	UMC2096	UMC2184	0.0004	0.0984
	UMC2112	UMC2142	0.0005	0.0911

Probint = Probability value for interaction Part R^2 = Partial R^2 value

Grain Yield

Single marker analysis detected four and eight markers that were significantly associated with grain yield in Weslaco and College Station, respectively (Table 4.16). The marker at position 2.03 (bnlg 2248) had the highest total phenotypic of 11% and it had significant positive additive effect (4.39**) while the marker at position 3.06 (umc1644) had the significant negative regression coefficient.

According to the likelihood ratio test statistics (LR) calculated by permutation, a total of seven QTLs were located in the different environments (Table 4.17, Figure 4.1). In Weslaco, two QTLs were detected in chromosomes 1 and 2 and they account for 10% and 9% of the phenotypic variation. In College Station, three QTLs were detected in chromosomes 1, 2, and 3 and these QTLs account for 9%, 14% and 7% of the total phenotypic variation.

In the across environment analysis, two QTL were detected in chromosomes 1 and 2 accounting for 12% and 12% of the phenotypic variation, respectively. Three putative QTLs were detected when the permutation LOD was lowered to 2.5. These QTLs were located in chromosomes 4, 9, and 3 and they account for 5%, 6% and 8% of the phenotypic variation.

The QTL with the largest effect was found in chromosome 2 with a total phenotypic variation of 14% and this was contributed by the CML161 parent. The total phenotypic variation for Weslaco, College Station and across both locations was 31%, 35% and 37%, respectively.

In this trait, thirty-eight digenic epistatic interaction were significant (Table 4.18) at P>0.001. In Weslaco, the interaction with the highest partial R^2 value was the interaction between unc1136 and umc1257 accounting for 12% of the total phenotypic variation, 21% in College Station and 10% in across locations after accounting for the main effects of he markers. Only 7 of the 38 digenic epistatic interactions involved loci which had

one locus with a significant main effect, one of the interactions involved loci which both had significant main effect. The remaining 33 interaction involved loci which both had no significant main effect.

Table 4.16 Markers single marker analysis significantly associated with grain yield in CML161xB73*o2* recombinant inbred line population in Weslaco and College Station

Location	Position	Marker	R^2	B73	CML161	Additive Effect
Weslaco	1.03	bnlg 2204*	0.04	23.28	26.72	1.72*
	1.03	Umc 2096*	0.04	23.26	26.95	1.84*
	2.07	Umc 1042*	0.04	23.39	26.89	1.75*
	2.08	Phi 127**	0.04	22.91	27.26	2.17**
College Station	1.04	Umc 1917*	0.03	21.02	25.62	2.29*
	1.03	Umc 2096**	0.06	19.89	25.81	2.96**
	1.03	bnlg 2204*	0.02	20.42	24.47	2.03*
	2.03	Umc 1555*	0.03	20.05	24.43	2.19*
	2.03	bnlg 2248**	0.11	17.3	26.08	4.39**
	3.06	Umc 1644*	0.04	25.51	20.62	-2.44*
	3.07	Txp 196 L*	0.03	25.54	20.91	-2.31*
	3.08	Txp 218 L*	0.03	25.39	20.84	-2.27*

* Significant at the 0.05 level of
** Significant at the 0.01 level

Table 4.17 Chromosome and map positions, nearest flanking marker loci, effects and variance associated with QTL identified in CML161xB73*o2* recombinant inbred line population in Weslaco, College Station and across locations for grain yield.

ENV	Chr	Pos	Lmarker	Bin	Rmarker	Bin	Lod	R^2	Add	FAllele
WE	1	76.7	umc 2096	1.03	umc1917	1.04	3.18	10	-2.72	B 73o2
	2	127.3	Phi127	2.08	umc1042	2.07	2.95	9	-2.62	B 73o2
	***4**	**66.5**	**umc2039**	**4.03**	**umc1652**	**4.04**	**2.27**	**5**	**2.17**	**CML 161**
	***9**	**5.9**	**bnlg1724**	**9.01**	**umc2393**	**9.01**	**2.29**	**6**	**-2.25**	**B 73o2**
CS	1	85.5	umc2096	1.03	umc1917	1.04	3.32	9	-3.77	B 73o2
	2	56.2	bnlg2248	2.03	umc1555	2.03	4.32	14	-4.65	B 73o2
	3	132.7	phi10222	3.06	umc1644	3.06	2.99	7	3.44	CML 161
Across	1	81.1	umc2096	1.03	umc1917	1.04	3.73	12	-2.90	B 73o2
	2	56.3	bnlg2248	2.03	umc1555	2.03	3.57	12	-2.90	B 73o2
	***3**	**40.7**	**bnlg1523**	**3.02**	**umc2258**	**3.03**	**2.27**	**8**	**-2.44**	**B 73o2**

Chr =chromosome, R-marker=Right marker,L-Marker =Left marker.
FAllele=Favorable Allele, Add =Additive effect, Pos=Position
Bold lettering indicates putative QTL according to lowered permutation LOD
R^2 indicates percentage of phenotypic variation explained (PVE)by the locus.

Table 4.18 Significant epistatic interactions between loci in CML161xB73*o2* recombinant inbred line population in Weslaco, College Station and across locations for grain yield.

Location	locus1	locus2	probint	partr2
Weslaco	TXP100L	BNLG1666	0.0006	0.0869
	BNLG1429	UMC1345	0.0001	0.1215
	BNLG1666	PHI114	0.0006	0.0899
	BNLG1805	UMC1703	0.0009	0.0780
	BNLG1953	UMC1345	0.0005	0.0951
	BNLG2191	UMC1912	0.0005	0.0943
	PHI024	UMC1644	0.0002	0.1081
	UMC1180	UMC2190	0.0009	0.0865
	UMC1231	UMC1257	0.0010	0.0796
	UMC1257	UMC1912	0.0009	0.0813
	UMC1345	UMC1976	0.0001	0.1145
	UMC1644	UMC2393	0.0008	0.0847
College Station	TXP100L	PHI127	0.0006	0.0834
	TXP100L	PHI21398	0.0006	0.0834
	TXP100L	PHI22756	0.0006	0.0834
	TXP100L	PHI23337	0.0006	0.0834
	TXP100L	PHI33188	0.0006	0.0834
	TXP100L	PHI37411	0.0006	0.0834
	TXP100L	PHI42070	0.0006	0.0834
	TXP100L	PHI45269	0.0006	0.0834
	TXP218L	BNLG1019	0.0009	0.0809
	BNLG1019	UMC1562	0.0002	0.1018
	BNLG1154	UMC2170	0.0005	0.0921
	BNLG1208	UMC2018	0.0006	0.0883
	BNLG1208	UMC2165	0.0009	0.0863
	BNLG1671	UMC1143	0.0005	0.0912
	BNLG1714	UMC1061	0.0007	0.0820
	BNLG1805	PHI072	0.0009	0.0841
	PHI024	UMC1639	0.0004	0.0964
	PHI072	PHI114	0.0006	0.0913
	PHI114	UMC1555	0.0007	0.0867
	UMC1506	UMC1966	0.0005	0.0933
Across	TXP100L	PHI072	0.0008	0.0861
	TXP100L	PHI127	0.0001	0.1014
	TXP100L	PHI21398	0.0001	0.1014
	TXP100L	PHI22756	0.0001	0.1014
	TXP100L	PHI23337	0.0001	0.1014
	TXP100L	PHI33188	0.0001	0.1014
	TXP100L	PHI37411	0.0001	0.1014
	TXP100L	PHI42070	0.0001	0.1014
	TXP100L	PHI45269	0.0001	0.1014
	TXP100L	UMC1042	0.0010	0.0877

Table 4.18 Continued. Significant epistatic interactions between loci in CML161xB73*o2* recombinant inbred line population in Weslaco, College Station and across locations for grain yield.

Location	locus1	locus2	probint	partr2
	TXP100L	UMC1161	0.0008	0.0898
	BNLG1019	UMC1562	0.0008	0.0864
	BNLG1666	PHI072	0.0007	0.0908
	BNLG1714	UMC2030	0.0003	0.0912
	BNLG2191	UMC1912	0.0007	0.0902
	BNLG2204	UMC1703	0.0009	0.0764
	BNLG2204	UMC2112	0.0005	0.0864
	PHI024	UMC1644	0.0003	0.0964
	PHI072	PHI114	0.0007	0.0891
	PHI114	UMC1161	0.0002	0.1115
	UMC1703	UMC2096	0.0009	0.0794
	UMC1917	UMC2165	0.0009	0.0866

Probint = Probability value for interaction. Part R^2 = Partial R^2 value.

Figure 4.1. Chromosomal locations of QTLs associated with Aflatoxin concentration and other secondary traits in 2005.

Discussion

Single marker analysis identified one marker umc1917 that was consistently associated with aflatoxin concentration in both College Station and Weslaco. Another marker bnlg1154 was significantly associated with logarithmic transformation of aflatoxin, which was also found associated with aflatoxin by Brooks et al. (2005).

Composite Interval analysis identified three QTLs in chromosome 1 and seven QTLs in for the logarithmic transformation of aflatoxin. Similar QTL's identified in chromosomes 3 and 5 by Paul et al. (2003) were also identified by Atta et al. (2006) but were flanked by different markers. Brooks et al. (2005) found QTLs associated with aflatoxin in chromosomes 1, 3, 5 and 6 but only two markers bnlg1953 and bnlg1154 flanking QTL's in chromosome 1 and 6 were similar to this study. Busboom and White (2004) detected QTLs for aflatoxin resistance in chromones 2, 3 and 7. One of the QTLs he detected in bin 3.08 was also detected in this study (Table 4.5)

A concentration of QTLs at single locations and across locations was located between markers bnlg2204, umc2096 and umc1917 in chromosome 1 (Figure 4.1). The largest QTL for both aflatoxin (ppb) and log (aflatoxin) explained 14% and 21% of the total phenotypic variation, respectively. Most of the QTLs controlling resistance to this complex trait were small effect QTLs. The influence of environment and genetics of this trait affects the accurate location of QTLs and the estimation of their effects. Paul et al (2003) also observed a number of QTLs with relatively small effect influencing aflatoxin concentration.

Resistance to aflatoxin was contributed by both parental CML161 and B73*o2*. Positive additive regression coefficient indicates lower aflatoxin was contributed from CML161 and negative regression coefficient effect indicates lower aflatoxin contributed from B73*o2* allele. This observation is consistent with reports by De Vicente and Tanksley

(1993) and Paul et al. (2003) that favorable QTLs come from both parents for a trait that appears to be under multigenic control.

Paul et al (2003) reports favorable aflatoxin QTL coming from the B73 parent. Several other QTLs for other secondary traits that were correlated with aflatoxin were identified in this study. Peterson et al. (1991) reported that correlated traits tend to have some of the same significant markers associated with the traits of interest. Traits that are correlated may have similar loci manifested through pleiotropy or linkage (Aastveit and Aastveit,1993). Based on Pearson's and genotypic correlations reported from the phenotypic data, aflatoxin was positively correlated to percentage of rotten ears and negatively correlated to grain yield. Negative genotypic and phenotypic correlations have earlier been reported by Betran et al. (2002). Based on QTL analysis and the correlation data, aflatoxin and grain yield are highly correlated and most of their QTLs were detected in similar genomic region.. This fact was buttressed by the identification of QTLs for aflatoxin and grain yield within same region in chromosomes 1 (Figure 4.1). A total of seven QTLs responsible for aflatoxin and grain yield were detected around markers Umc2096 and Umc1917 (Figure 4.1). It could be that these QTLs conditioning the different traits maybe linked.

Several of the QTLs for grain yield were large effect QTLs which were detected in different locations. The positive additive effects resulted in high value for yield corresponding to CML161 allele (Table 4.16) while negative additive effects results in high value for yield corresponding to B73*o2* allele, although favorable QTLs were found to be contributed by both parents. This is not strange for a quantitative trait as yield that appears to be under multigenic control.

Another secondary trait is endosperm texture, which is also correlated to aflatoxin. Betran et al. (2002) reported that aflatoxin was positively correlated to texture ratings. Even though these traits were not significantly correlated, five QTLs corresponding to

aflatoxin, grain yield and endosperm texture were deleted between phi046 and bnlg1047 (Figure 4.1).

Endosperm texture had eleven QTLs, which is the highest number of QTLs detected in the overall study for a single trait. Ten of the QTLs had positive regression coefficient corresponding to a low value for texture rating (i.e., flinty endosperm) from CML161 (Table 4.14). Although three QTLs having a negative regression coefficient which corresponds to the B73*o2* allele, only one of them accounted for 10% of the phenotypic variation of 10%.

Percentage of rotten ear is a secondary trait that is correlated to aflatoxin concentration. Betran et al. (2004) reported that aflatoxin accumulation in yellow and white hybrids were positively correlated to ear rot. The CML161 alleles were responsible for lower percentage of rotten ears at the two QTLs identified for this trait.
There were no observed correlation between aflatoxin and maturity. This observation is similar to earlier findings by Betran et al. (2002) that there was significant correlation between maturity measured as silking date and aflatoxin. All the QTLs identified for this trait were the small effects.

There were thirty eight significant epistatic interaction observed for aflatoxin in this study. There has been little or no report on the importance of epistasis in determining QTLs for aflatoxin resistance in maize. It was interesting to note the amount of significant epistatic interaction observed by testing interactions between all loci not regarding their individual effects.

Among the six traits involving 220 significant epistatic interactions, only 2 interactions involved loci which both had significant main effects. One of such was between umc1147 and umc 2094 for maturity. The other was for grain yield and the loci was umc 1644 and umc 2393. However, 38 involved loci which one locus had a significant main

effect. The remaining 180 interactions involved loci which both had no significant main effect.

This shows that several significant epistatic interactions would not have be found if we had not examined interactions between all loci regardless of their significance. This finding on QTL on epistasis may play a notable role in marker assisted selection. Analysis using near isogenic lines could further explain the interactions involving only one significant main effect. Holland et al (1997) explained that the effects of QTLs that exhibit interactions with unlinked genes may be altered dramatically when they are incorporated into a genetic background different from the one in which they were mapped.

The results observed in this study were consistent with those of Paul et al. (2003) and Brooks et al. (2005) in terms of location of QTLs in chromosome 1 and the large number of small effect QTLs affecting aflatoxin. The information obtained from this study on the location and estimation of QTLs for aflatoxin and its secondary trait will enable breeders to incorporate them into susceptible inbred through the use of marker assisted selection. Since breeding for aflatoxin resistance in a conventional way is often complicated by genotype by environment interactions and costly phenotyping, the QTLs identified here will be useful in reducing aflatoxin levels when transferred into other genetic background. Bernardo (2001) reported that it is better to make selection on basis of detected QTLs when there are few loci affecting the trait. Holland (2004) suggested that marker assisted selection is more efficient when the few QTLs with moderate and consistent effects across environments and breeding populations are detected. Robertson et al. (2005) reported that the number of loci conditioning a trait influences the potential efficiency of marker assisted selection.

The identification of QTLs for aflatoxin and its secondary correlated traits within the same chromosome region also reveals that these correlated traits QTLs could be useful in the marker assisted selection of aflatoxin.

The QTLs observed in this study could be validated by testing in different genetic backgrounds, different locations and years. The identified QTL could be used in several ways, one is that they could be incorporated into susceptible inbred through the use of marker assisted selection another is that they may play an important role in finding candidate genes that is useful in explaining the genetics of aflatoxin resistance. Lastly is a tool that has been used to understanding the genetic relationship between correlated traits .

CHAPTER V
CONCLUSIONS

EXPERIMENT 1: GENERATION MEAN ANALYSIS OF AFLATOXIN ACCUMULATION

The distribution of aflatoxin means showed skewness towards the resistant parent in all six crosses. Also aflatoxin accumulation was greatest in College Station in all six crosses. Additive and dominant effects were significant in this group of crosses. Inbred CML176 and CML161 and their generations showed reduced levels of aflatoxin, suggesting that it has resistant factors that are heritable. It is concluded that they can be used as sources of resistance for selection and mapping in breeding programs.

EXPERIMENT 2: PHENOTYPIC CHARACTERIZATION OF A RECOMBINANT INBRED LINE MAPPING POPULATION CML161 X B73o2

Heritability estimates for aflatoxin and its secondary traits such as endosperm texture, percentage of rotten ears and grain yield were moderate to high. Higher heritability estimates were observed in Weslaco than in College Station. Aflatoxin was positively correlated with endosperm texture and percentage of rotten ears but negatively correlated with grain yield. There was no observed correlation between aflatoxin and maturity. There is a great potential for genetic gain for traits with high heritability and significant genetic correlations to aflatoxin as it could be used in indirect selection.

EXPERIMENT 3:GENETIC MAPPING AND ANALYSIS OF QUANTITATIVE TRAIT LOCI IN A MAIZE RECOMBINANT INBRED LINE POPULATION

Single marker analysis identified one marker Umc1917 that was consistent associated with aflatoxin concentration in both College Station and Weslaco. Composite interval analysis identified three QTLs in chromosome 1 for aflatoxin explaining 41% and 40% of the total phenotypic variation in Weslaco and College Station, respectively. Seven QTLs were identified in chromosomes for logarithmic transformation of aflatoxin

explaining 38%, 55% and 38% of the total phenotypic variation in Weslaco, College Station and across locations, respectively. A concentration of QTLs was observed between markers bnlg2204, Umc2096 and Umc1917 in chromosome 1. Most of the QTLs controlling resistance to aflatoxin were small effect QTLs and contributed by both parents CML 161 and B73*o2* Some of the QTLs that were putative QTLs with aflatoxin (ppb) became real QTL with log transformation of aflatoxin. Approximately 55% of the QTLs found in the across environment were also found in the single environment and they were similar in parental contribution and magnitude of their additive effect.

The identification of QTLs for aflatoxin and its secondary correlated traits within the same chromosome region also reveals that these correlated traits QTLs could be useful in the marker assisted selection of aflatoxin. The QTLs detected in this study are promising and may be confirmed in other environments and breeding population for subsequent use in marker assisted selection.

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