THE EFFICACY OF MARKER-ASSISTED SELECTION FOR GRAIN MOLD RESISTANCE IN SORGHUM

A Dissertation

by

CLEVE D. FRANKS, JR.

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

December 2003

Major Subject: Plant Breeding

THE EFFICACY OF MARKER-ASSISTED SELECTION FOR GRAIN MOLD RESISTANCE IN SORGHUM

A Dissertation

by

CLEVE D. FRANKS, JR.

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

DOCTOR OF PHILOSOPHY

Approved as to style and content by:

William L. Rooney (Chair of Committee)	Gary N. Odvody (Member)
Robert R. Klein (Member)	Darrell T. Rosenow (Member)
Mark A. Hussey (Head of Department)	

December 2003

Major Subject : Plant Breeding

ABSTRACT

The Efficacy of Marker-Assisted Selection for Grain Mold Resistance in Sorghum.

(December 2003)

Cleve D. Franks, Jr., B.S., California Polytechnic State University;

M.S., Texas A&M University

Chair of Advisory Committee: Dr. William L. Rooney

Five breeding populations were created by crossing elite U.S. sorghum parental lines (RTx430, RTx436, BTx631, BTx635, and Tx2903) with 'Sureño', a dual purpose grain mold resistant sorghum cultivar. Molecular markers associated with five previously-reported quantitative trait loci (QTL) for grain mold resistance originating in 'Sureño' were used to determine if their presence enhanced selection for grain mold resistance in these populations. The allelic status of 87 F_4 lines, with respect to these QTL, was determined using both simple sequence repeats (SSR) and amplified fragment length polymorphism (AFLP) markers. All 87 $F_{4:5}$ lines and their parental lines, were evaluated for grain mold resistance in replicated trials in eight diverse environments in South and Central Texas during the summer of 2002. The effects of each allele from the grain mold resistant parent 'Sureño' were determined across and within all five populations, within individual environments, and in each population x environment combination. With a few exceptions, the QTL were effective in reducing grain mold susceptibility only within the RTx430/Sureño progeny, the identical cross that was used

in the original mapping study. The results indicate that while that these alleles do confer additional grain mold resistance, they are only selectable in the original mapping population. This fact limits their potential usefulness in an applied breeding program.

DEDICATION

I dedicate this work to my God and my family, who have provided me with the inspiration needed to see it through.

ACKNOWLEDGEMENTS

I would like to first of all thank Dr. Bill Rooney, for all of his help and patience throughout this process. I have truly been fortunate to have him as an advisor and friend.

I also owe a debt of gratitude to all of my fellow graduate students in the Sorghum Breeding Program: Jorge, P.J., Adalberto, Rafael, Gabriel, Les and Joaquin. Thanks for all the help and friendship.

This dissertation could not have been completed without the generous assistance of the technical staff of the A&M Sorghum Research Group. Many thanks to Delroy Collins, Cindy King, Karen Prihoda, Natalie Unruh, Julie McCollum, and student workers over the years too numerous to mention.

TABLE OF CONTENTS

LIST OF TABLES

TABLE Page

TABLE Page

TABLE Page

LIST OF FIGURES

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most widely cultivated grain crop in the world (Food and Agriculture Organization of the United Nations, 2003). Sorghum is used as a staple food grain in developing countries and as a feed grain in developed nations. United States production estimates for the year 2002 place the total yearly crop at 370 million bushels, grown on nearly 7.3 million acres (National Agricultural Statistics Service, 2003). Sorghum is drought and heat tolerant and it can produce a harvestable crop in environmental extremes where most other cultivated grain crops cannot. Increasing world population and its consequent demands on both land and water resources ensure that sorghum will remain an important crop in the future.

Sorghum evolved and was domesticated in Northeast Africa near the equator, where sorghum cultivars are effectively able to avoid late season rains by postponing flowering until the dry season (Williams and Rao, 1981). The mechanism by which the plants accomplish this is through photoperiod sensitivity, in which the plant switches from vegetative to reproductive growth in response to a narrowly defined daylength. For commercial grain production within the U.S., this is an undesirable characteristic, as photoperiod-insensitive hybrids suitable for combine harvesting are required for these production environments. To meet this need, plant breeders in more temperate environments have selected early-maturing, photoperiod-insensitive plant types.

This dissertation follows the style and format of *Crop Science*.

As sorghum was bred for use in higher production agricultural systems and introduced to other regions, it was exposed to environmental conditions which were not found within its areas of domestication. The exposure of mature grain to late season rains increases the incidence and severity of both pathogenic and saprophytic fungi on the seed in the panicle. Collectively, these fungi comprise the disease known as grain mold of sorghum.

Resistance to grain mold of sorghum has been a difficult trait to incorporate into elite germplasm. Because of this difficulty, molecular marker technology may provide a much-needed method of enhancing the plant breeder's ability to select lines with increased levels of resistance. Previous work by Klein et al. (2001) paved the way for such a study by identifying quantitative trait loci (QTL) associated with enhanced grain mold resistance in a recombinant inbred line population. Ongoing mapping projects since this study have increased the precision with which these loci can be distinguished by further expanding the arsenal of molecular markers available.

The objectives of this study were to:

1. Determine the efficacy of the previously defined grain mold QTL across a range of adapted sorghum germplasm in widely varying environments.
2. Determine which, if any, of the QTL were effective in predicting the grain mold response of the progeny lines.
3. Develop a set of adapted sorghum germplasm with increased grain mold resistance to grain mold effective across an array of environments.

LITERATURE REVIEW

I. Grain Mold of Sorghum

Researchers often delineate grain mold from grain weathering, based upon the physiological stage at which infection and colonization of mold-producing fungi occurs (Castor and Frederiksen, 1980; Forbes et al., 1992). Typically, mold infection prior to physiological maturity is caused by pathogenic fungi invading the grain from the germ, and is referred to as grain mold. After physiological maturity, saprophytic fungi, which invade the kernel from the tip and progress downward, are said to cause grain weathering. These distinctions between grain mold and weathering can be important in terms of the precise type of damage caused and the particular fungal species which cause them. However, from a practical standpoint, breeding for mold resistance must be successful for both phenomena. For the purposes of this study, both grain mold and weathering are being considered as one phenomenon.

The symptoms of grain mold vary according to the particular fungal species present, the stage of plant growth and level of susceptibility of the plant host, and the favorability of the environment for establishment of the disease. Certain characteristic symptoms, however, are normally apparent when grain mold is present. Discoloration of the grain, as well as pink or black fungal sporulation on the surface of the grain, is perhaps the most common and immediately observable symptom (Castor and Frederiksen, 1980; Williams and Rao, 1981). Other frequently observed symptoms include preharvest sprouting, reduced germination and seedling vigor, reduced seed

weight, size, and endosperm and, less frequently, the presence of mycotoxins within the harvested grain (Castor and Frederiksen, 1980). Reductions in yield, although difficult to quantify, also accompany severe grain mold infections.

It is often difficult to identify which fungal species is the primary causal agent for a given situation where grain mold occurs. Over 30 genera of fungi have been associated with sorghum grain mold and weathering (Williams and Rao, 1981). Even in environments in which grain mold pressure is mild, a myriad of fungi can be readily isolated from grain produced in the field. Many of these organisms, however, are merely incidental to field conditions and do not directly contribute to the symptoms of grain mold to a significant degree (Castor and Frederiksen, 1980). Studies attempting to identify specific pathogens that are causal agents of grain mold typically identify a handful of fungal genera: *Fusarium, Curvularia, Alternaria, Aspergillus, Cladosporium*, and *Phoma* (Bandyopadhyay and Mughogho, 1988; Canez and King, 1981; Castor and Frederiksen, 1980; Williams and Rao, 1981). Castor and Frederiksen (1980) identified *Fusarium moniliforme, F.semitectum, Curvularia lunata, C. protuberata* and *C. trifolii* as the principal agents responsible for grain mold damage in Texas.

Measures for controlling grain mold are limited. Cultural methods include planting either earlier maturing or late maturing hybrids and harvesting the grain in a timely fashion, but these measures are not always practical nor effective (Bandyopadhyay et al., 2002). While chemical control can be effective, it is not economically feasible. For these reasons, genetic resistance remains the only economically viable means to reduce grain mold on sorghum.

II. The Genetics of Resistance to Grain Mold of Sorghum

The underlying genetic mechanisms governing the resistance of sorghum to grain mold are poorly understood. Grain mold resistance in sorghum is a complexly inherited trait further complicated by the fact that many simply inherited, kernel-based traits influence the level of grain mold resistance found in a particular genotype. Some of the kernel characteristics reported to enhance grain mold resistance are a pigmented testa, a red pericarp, a thin pericarp, corneous endosperm, increased flavan-4-ol content, reduced water uptake capacity in mature grain, open panicle structure, and taller plants (Bandyopadhyay and Mughogho, 1988; Esele et al., 1993; Glueck and Rooney, 1980; Harris and Burns, 1973; Ibrahim et al., 1985; Menkir et al., 1996; Rodriquez et al., 2000; Waniska et al., 1989). Recent studies examining the role of antifungal proteins in grain mold resistance suggest that these compounds may also play an important role in the inhibition of grain mold of sorghum (Waniska et al., 1992). While all these traits affect grain mold resistance, none of these characteristics, either singly or in combination, guarantees that a line will possess resistance to grain mold, nor does their absence assure that a line will be susceptible to grain mold (Glueck and Rooney, 1980). Moreover, several of these characteristics (particularly a pigmented testa and increased levels of tannins) are undesirable from the perspective of feed grain producers.

In addition to kernel-based traits, there appears to be a basic quantitative genetic level of resistance to grain mold in sorghum. Studies done on both the heritability of grain mold resistance and estimates of the number of genes involved clearly point to the fact that grain mold resistance is a quantitative trait. Rodriguez-Herrera et al. (1999),

using a population derived from parents without a pigmented testa, estimated the broad sense heritability of grain mold resistance to be between 0.49 and 0.82, while narrow-sense heritability estimates ranged between 0.39 and 0.59. The same study estimated that between four and 10 genes contribute to grain mold resistance. Dabholkar and Baghel (1983) estimated the narrow-sense heritability of grain mold resistance to be between 0.04 and 0.12, and found both additive and nonadditive gene effects in grain mold resistant lines. Murty et al. (1988) indicated that the variation for resistance to grain mold within populations segregating for grain mold resistance was continuous, a characteristic indicative of quantitative traits. It is axiomatic that numerous genes control quantitative traits, each impacting the trait in an almost phenotypically imperceptible way, and that environmental conditions have a profound effect on the expression of the trait.

Despite the complexity of the inheritance of grain mold resistance and the associated difficulty of breeding for grain mold resistance, some measure of success has been realized in this endeavor. However, in a typical breeding program, numerous traits are selected simultaneously, and the addition to such a program of even a simply inherited trait can substantially reduce the likelihood of effectively selecting breeding lines superior for the other traits under consideration. It is therefore desirable that tools be developed which would offer plant breeders the opportunity to effectively select for those particular genes which have the greatest positive impact. If successful, this approach could potentially diminish the arduous task of testing excessively large

numbers of breeding lines in multiple locations and years in the hope that favorable genic combinations can be discovered.

III. Molecular Mapping and Markers

There has been a dramatic increase in the number and variety of molecular markers available to researchers in recent years. Isozyme and RFLP-based markers have largely given way to PCR-based molecular markers, which are greater in number and easier to score. Currently, the most powerful and prevalent among these PCR-based markers are simple sequence repeats (SSR, or microsatellites), and amplified fragment length polymorphisms (AFLP). SSR markers distinguish individuals based upon the number of tandem repeats of a given simple nucleotide sequence (Hamada et al., 1982). Specific primers flanking the region in which the repeats occur are designed, and the amplified DNA is separated and visualized via electrophoresis, so that these differences in repeat number can be distinguished. Many SSR markers have the advantage of being co-dominant, meaning that heterozygotes for the allele in question can be detected. AFLP involves the digestion of a DNA template with specific restriction enzymes, followed by the ligation of specially-designed adapters onto the sticky ends of the sample DNA (Vos et al., 1995). A subset of the available DNA strands is then amplified by extending the adapter-based primers by a single base pair into the template strand. Thereafter, primers with specific three base pair additions to the adapter-based primers are used in combination to amplify a very limited subset of DNA strands which contain polymorphisms based on restriction site, primer recognition, or addition/deletion events

within the amplified region. Thus, with a limited number of primer combinations, a multitude of AFLP markers can be generated efficiently and rapidly. Fluorescently labeled nucleotides incorporated during PCR allow multiplexing of primers via visualization at different wavelengths. Depending upon the nature of the polymorphism, AFLP markers can be either dominant or codominant.

The development of newer, more prolific molecular marker systems has consequently led to an increase in the number and quality of molecular maps for virtually all plant species of economic importance. Sorghum researchers have enjoyed the results of this expanding technology through the development and publication of numerous linkage maps (Chittenden, et al., 1994; Kong et al., 2000; Menz et al., 2002; Peng et al., 1999; Pereira et al., 1994; Rami et al., 1998; Xu et al., 1994). Typically, molecular maps are made utilizing progeny developed from crosses between adapted and exotic parents, so that the number of useful polymorphisms is maximized. This strategy is employed by geneticists to produce a more saturated map. While this approach does indeed result in a higher density of markers, the resultant map may be of limited use to breeders, who do not normally utilize as diverse a set of germplasm in their programs.

Klein et al. (2001) published a molecular map which identified five QTL, each explaining between 10 and 24 percent of the observed variation for grain mold resistance within recombinant inbred sorghum lines derived from a RTx430/Sureño cross. Consistent with the qualities of quantitative traits, the effects of the QTL varied across environments. The map constructed by Klein et al. differs somewhat from typical molecular maps with respect to the population structure used in its development. While

recombinant inbred lines (RILs) were utilized to construct the map, the initial parental lines chosen to create the cross could both be considered elite germplasm. RTx430 is a widely used restorer line with exceptional combining ability and adaptation, and is commonly used in the production of hybrid sorghum in the U.S (Miller, 1984). With the exception of its tall height, Sureño can likewise be classified as elite, possessing all of the qualities desirable in commercial grain sorghum production (Meckenstock et al., 1993). In principle, therefore, markers observed as polymorphic between these two lines should be more readily applicable to breeding populations. This map was used as the basis for this study, which aimed to corroborate the work of Klein et al. (2001), and to further test the efficacy of the grain mold resistance QTL in a broader array of germplasm and environments.

IV. Marker-Assisted Selection

One of the principal motivations behind the creation of molecular maps is their potential application in marker-assisted selection (MAS). The identification of markers associated with a trait of interest would allow the informed introgression of the gene (or genes) that code for that trait, irrespective of the phenotypic expression of the trait itself. Marker-assisted selection is a form of indirect selection which essentially allows the breeder to select for the genotype of a given plant or breeding line, instead of the variable phenotype. The potential strength of MAS lies in its ability to detect agronomic traits that are otherwise difficult to observe phenotypically (Mohan et al., 1997). Resistance genes have frequently been the proposed targets of such efforts, since the

pyramiding of resistance genes to multiple races of a particular pathogen is often difficult. In other cases, screening for resistance to a pest or pathogen is time-consuming or expensive, while the PCR-based evaluation of lines for the presence or absence of a single marker is comparatively easy. The improvement of quantitative or oligogenic traits is another possible area in which MAS may be useful. The large influence of the environment upon these traits, as well as their small *per se* phenotypic effects makes successful selection for them challenging, and, in some cases, impossible. Traits that are monogenic in inheritance are usually quite easy for breeders to select for, but the elucidation of the underlying genetic mechanisms and inheritance of quantitative traits has been a long-awaited development by plant breeders.

The identification of markers associated with these traits is a prerequisite for their targeted introgression using MAS. The development of powerful mapping software has greatly increased the precision with which these marker/QTL associations can be detected. The advancement in marker technology has enabled geneticists to map these traits with much greater levels of power and precision than were previously possible using RFLP or isozyme markers (Mohan, et al., 1997). In addition, the simplification of marker systems and DNA extraction protocols has brought the prospect of using these systems on the large numbers of genotypes inherent in plant breeding programs into the realm of feasibility.

Despite the development of numerous molecular maps and the increased ease and efficiency of marker systems, there has been limited success of marker-assisted selection in applied breeding programs (Young, 1999). There remains concern over the

applicability of using markers in the selection of QTL, and validation of the theory of marker-assisted selection in these cases is yet to be reported. Most cases of reported success with marker-assisted selection have involved the use of markers tightly linked to genes controlling qualitative traits, such as resistance to soybean cyst nematode or rice blast (Hittalmani, et al., 2000; Huang, 1997; Young, 1999). The need to test these strategies within the context of an applied breeding program was one of the principal motivations behind the current investigation.

MATERIALS AND METHODS

I. Germplasm and Population Development

Sureño, a dual-purpose sorghum variety with exceptional resistance to grain mold, was crossed, via hand emasculation, to each of the following adapted U.S. parental lines: RTx430, RTx436, BTx631, BTx635, and Tx2903 (Meckenstock, et al. 1993; Miller, 1984; Miller, 1986; Miller et al., 1992(1); Miller et al., 1992(2); Miller and Prihoda, 1996) (Table 1). The variety Sureño was originally selected from an ICRISAT-developed line, via Mexico (CIMMYT), and identified in the Texas Agricultural Experiment Station Grain Weathering Test (GWT). The original source was reselected in Texas and entered into the TAES GWT. It was then identified in the GWT in Honduras and subsequently released in Honduras as a dual-purpose variety for its high quality grain and forage. All of the adapted parents were released from of the Texas Agricultural Experiment Station Sorghum Breeding Program, and are representative of the range of lines currently used in U.S. hybrid sorghum. After the F_1 generation was self-pollinated, F_2 plants were selected for dwarf plant type, and seed from each self-pollinated panicle was advanced to the next generation. In the subsequent generation, 545 $F_{2:3}$ lines were planted in three locations in Texas: Beeville, College Station, and Weslaco, with two replications in each environment. At each location, the lines were evaluated for an array of agronomic traits, and 87 lines were selected for advanced testing. The lines were advanced using a single F3 plant which had been self-pollinated. Selections were based on both grain mold resistance and overall agronomic desirability.

Traits considered in making selections included plant height, panicle exsertion, plant color, grain color, grain mold rating, and overall agronomic desirability. Plant height was measured as the height from the soil line to the tip of the panicle while panicle exsertion was measured from the base of the flag leaf to the tip of the panicle. Plant color was either pigmented or tan (relevant only for Sureño/RTx430 progeny), and grain color was recorded as either white or red (relevant only for Sureño/Tx2903 progeny). Grain mold reaction was scored using a modified version of the scheme established by Frederiksen, et al. (1991): a rating scale of one to nine was used where a rating of one indicated that the seed was entirely free from mold damage and a score of nine represented completely deteriorated grain. All grain mold ratings were taken approximately 6-9 weeks after physiological maturity. Irrespective of the stage at which infection and subsequent molding occurred we refer to both diseases mentioned above as "grain mold". All traits were scored in the field and no attempt was made to rate harvested or threshed grain. Agronomic desirability was measured on a scale on a scale of 1 to 9, with 1 representing the most agronomically desirable line, and 9, the least.

Of the 87 lines advanced, approximately equal numbers of $F_{3:4}$ lines from each of the five populations were advanced (Table 1). These F_4 lines were grown in Weslaco, Texas in the fall of 2001, where a single F_4 plant was randomly selected for the purpose of non-destructive DNA extraction and 10 panicles on phenotypically similar plants were self-pollinated. Seed from these plants were harvested and bulked to produce the F_5 seed used in the replicated testing of grain mold resistance in these lines. The DNA sample was considered representative of that line.

Table 1. Parental lines used in population development and marker assisted selection.

Line	Pedigree	Year of Release	Epicarp Color	Plant Color	Inbred Lines
Sureño	[(SC423*CS3541)*E35-1]-2	1993	W	T	
RTx430	(Tx2536*SC170-6-5-1-E_2)-10-4-4-1-4-⊗	1984	W	P	12
RTx436	(SC120-6-sel/2*Tx7000)-10-4-6-1-1-1-bk	1992	W	T	19
BTx631	((BTx378*SC110-9)*BTx615)-4-3-5-2-1-2-⊗-⊗	1986	W	T	17
BTx635	RS/R (C2) S_1 102-1-1-2-1-5-1	1992	W	T	17
Tx2903	{[(SC120-6*Tx7000)*Tx7000]-10-2-6-2-CBK*Tx433}F2-B13-B1-B1-B3-B1-B3-CBK	1996	R	T	22

II. Field Evaluation

In the summer of 2002, 87 $F_{3:5}$ lines were evaluated at six locations across Texas in randomized complete block trials with two replications per location. The remaining 22 lines either did not produce enough seed or were eliminated due agronomic or grain mold problems. In all replicated tests the six parental lines were included as checks. The trials were grown in Beaumont, Beeville, College Station, Corpus Christi, Victoria,

and Weslaco. In College Station and Corpus Christi, two separate trials were conducted in fields at least one km in distance apart (Table 2). Inclusion of these trials resulted in a total of eight environments. Phenotypic traits recorded in this trial included plant height, panicle exsertion, plant color, grain color, days to mid-anthesis, and subjective ratings for agronomic desirability and grain mold.

Many field-based sorghum grain mold studies have utilized inoculations, bagging of heads, sprinkler irrigation, or combinations thereof to ensure an adequate level of mold pressure for the purposes of scoring resistance. Late season rains, high humidity, and predictably high temperatures are normal in the test environments included in this study, however, and previous studies and breeding trials in these locations had indicated that ample grain mold levels could be expected in any given year (Castor and Frederiksen, 1980, Klein et al., 2001, Rodriguez-Herrera et al., 2000). Therefore, it was decided in advance to rely upon natural inoculum and not include artificial inoculation techniques or methods of enhancing grain mold pressure in this study.

Table 2. Dates of planting and observations, related to mean maturity of nursery.

	Planting Date	Mean Number of Days to Mid-Anthesis	Observation Date	Days After Mid-Anthesis
Beaumont	3/28/02	82.4	8/07/02	50
Beeville	3/13/02	86.8	7/19/02	41
Corpus Christi (Annex)	3/12/02	83.0	7/20/02	47
Corpus Christi (Station)	3/06/02	86.8	7/20/02	50
College Station (North)	3/28/02	81.1	8/17/02	61
College Station (South)*	4/05/02	-	8/25/02	-
Victoria*	3/15/02	-	7/27/02	-
Weslaco	2/14/02	80.1	6/24/02	49

* Days to mid-anthesis were not recorded in these locations.

III. Molecular Marker Analysis

Plant tissue harvested from F_4 plants at the seedling stage for the purposes of DNA extraction was stored in dry ice prior to DNA extraction. All marker work was done using DNA from these plants. In order to screen markers for polymorphism,

original parental seed sources for all crosses were obtained from the Texas Agricultural Experiment Station Sorghum Breeding Program. These seeds were planted in a greenhouse and tissue was sampled for DNA extraction.

DNA was extracted using Bio 101 Fastprep extraction kits (Qbiogene, Inc., Carlsbad, CA). All DNA samples were quantitated via fluorimetry using Hoechst dye as a buffer.

Since there were five distinct populations in this study, it was clear that there would be varying degrees of polymorphism between Sureño and the respective adapted parents. To maximize the number of markers available for this study, the map constructed by Klein et al. (2001) was compared with the web-based sorghum genome map of Menz et al. (2002). Molecular markers linked to the sorghum grain mold QTL identified by Klein et al. were screened for polymorphism using DNA from the parental lines. Those markers which proved polymorphic between Sureño and any of the adapted parents were subsequently amplified, visualized, and scored in the respective populations. Both SSR and AFLP markers were utilized in this study, and the method of visualization depended upon both the particular marker system and the nature of the polymorphism itself.

For the purposes of SSR work, DNA template was diluted to a concentration of 2.5ng/µl and arrayed in 96-well microplates for ease of reaction setup. Forward and reverse SSR primers at 1 Pm/µl were added to a PCR master mix consisting of 1x Promega PCR buffer, 2.5 Mm $MgCl_2$, 2Mm dNTPs, and 0.4U Promega Taq polymerase (Promega, Madison, WI). Amplification profiles for all were as follows : an initial

denaturation of template DNA at 94˚C for 2 min 30 seconds, followed by 33 cycles of 94˚C for 1 min, 55˚C for 1 min, and 72˚C for 1 min. The final annealing step was 72˚C for 10 min.

For a given SSR marker under consideration, an initial PCR was run with DNA from all parents and visualized via super fine resolution agarose gel (4.5%, stained with ethidium bromide). If a polymorphism between a particular parent and Sureño was visible using agarose gels, then the progeny of that cross were amplified and likewise run on agarose gels. If there was no polymorphism visible, or the polymorphism was not easily scored with agarose, then the reaction was rerun and separated on a 5% polyacrylamide gel. Silver staining, as described by Fritz, et al. (1995), was used to visualize DNA migration on all polyacrylamide gels. All AFLP markers were visualized using the LiCor gel system.

IV. Data Analysis and Models

To analyze the differences in grain mold resistance among the original parental lines, the following model was used: $y_{ijk} = \mu + P_k + L_i + R_{ij} + (L*P)_{ik} + \varepsilon_{ijk}$, where

y_{ijk} = the grain mold rating of a given parental line;

μ = overall mean;

P_k = response due to k^{th} adapted parent;

L_i = response due to i^{th} environment;

R_{ij} = response due to j^{th} replication within i^{th} environment;

$(P*L)_{ik}$ = response due to interaction between i^{th} environment and

k^{th} adapted parent; and

ε_{ijk} = random error term associated with a given observation.

Appropriate tests of significance were based on expected mean squares where replications and environments were considered random effects, while parents were treated as fixed effects (Table 3).

Table 3. Expected mean squares of analysis of variance, parental lines.

Source	EMS
Parents	$\sigma_e^2 + lr\delta_P^2 + r\delta_{PL}^2$
Locations	$\sigma_e^2 + p\sigma_{R(L)}^2 + pr\sigma_L^2$
Replications(Locations)	$\sigma_e^2 + p\sigma_{R(L)}^2$
Parents x Locations	$\sigma_e^2 + r\delta_{PL}^2$
Error	σ_e^2

The model used in examining the F_5 progeny of the respective parental lines and the environments (apart from the effects of the QTL altogether) was:

$y_{ijkl} = \mu + L_i + R_{ij} + P_k + G_{kl} + (L*P)_{ik} + \varepsilon_{ijkl}$, where

y_{ijkl} = the grain mold rating of a given individual;

μ = overall mean;

L_i = response due to i^{th} environment;

R_{ij} = response due to j^{th} replication (nested within i^{th} environment);

P_k = response due to k^{th} adapted parent;

G_{kl} = response due to l^{th} genotype (nested within k^{th} adapted parent);

$(L*P)_{ik}$ = response due to interaction between i^{th} environment and k^{th} adapted parent; and

ε_{ijkl} = random error term associated with a given observation.

Appropriate tests of significance were based on expected mean squares where replications and environments were considered random effects, while parents were treated as fixed effects (Table 4). This model was used strictly to determine the effects of parent, environment, and their interactions, upon the plant characteristics recorded.

Table 4. Expected mean squares of analysis of variance for environments and F_5 progeny.

Source	EMS
Parents	$\sigma_e^2 + lrg\delta_P^2 + lr\sigma_{G(P)}^2 + gr\delta_{PL}^2$
Genotypes(Parents)	$\sigma_e^2 + lr\sigma_{G(P)}^2$
Locations	$\sigma_e^2 + pg\sigma_{R(L)}^2 + pgr\sigma_L^2$
Replications(Locations)	$\sigma_e^2 + pg\sigma_{R(L)}^2$
Parents x Locations	$\sigma_e^2 + gr\delta_{PL}^2$
Error	σ_e^2

The model used to analyze the effects of particular QTL, both across and within parental lines was:

$y_{ijkl} = \mu + L_i + R_{ij} + Q_m + (L*Q)_{im} + \varepsilon_{ijm}$, where

y_{ijm} = the grain mold rating of a given individual;

μ = overall mean;

L_i = response due to i^{th} environment;

R_{ij} = response due to j^{th} replication within i^{th} environment;

Q_m = response due to m^{th} allele;

$(L*Q)_{im}$ = response due to interaction between i^{th} environment and

m^{th} allele; and

ε_{ijm} = random error term associated with a given observation.

In this analysis, the effects of each of the five QTL were analyzed separately across environments, without consideration of the potentially different responses of the parents to a given QTL. Environments and replications were considered random effects, while the two possible classes of QTL status were fixed (Table 5).

Table 5. Expected mean squares of analysis of variance for QTL, across parents and environments.

Source	EMS
Locations	$\sigma_e^2 + q\sigma_{R(L)}^2 + rq\sigma_L^2$
Reps(Locations)	$\sigma_e^2 + q\sigma_{R(L)}^2$
QTL	$\sigma_e^2 + r\delta_{LQ}^2 + lr\delta_Q^2$
Location x Allele	$\sigma_e^2 + r\delta_{LQ}^2$
Error	σ_e^2

To analyze the effects of QTL within each environment (treating all of the observations from a particular environment as a unique and independent dataset), the following model was used:

$y_{kjm} = \mu + P_k + Q_m + (P*Q)_{km} + R_j + \varepsilon_{kjm}$, where

y_{kjm} = the grain mold rating of a given individual;

μ = overall mean;

P_k = response due to k^{th} parent;

R_j = response due to j^{th} replication;

Q_m = response due to m^{th} allele;

$(P*Q)_{km}$ = response due to interaction between k^{th} parent and m^{th} allele; and

ε_{kjm} = random error term associated with a given observation.

Test of significance were based on a mixed model where parents and quantitative trait loci were considered as fixed factors, while replications were treated as random factors (Table 6).

Table 6. Expected mean squares of analysis of variance for QTL, within environments.

Source	EMS
Parents	$\sigma_e^2 + rq\delta_P^2$
Replications	$\sigma_e^2 + pq\sigma_R^2$
QTL	$\sigma_e^2 + pr\delta_Q^2$
Parent x QTL	$\sigma_e^2 + r\delta_{PQ}^2$
Error	σ_e^2

Finally, each parent-environment combination was analyzed separately to evaluate the effect of each particular grain mold QTL within a population in a given environment. The model for this analysis of variance was:

$$y_{mk} = \mu + Q_m + R_k + \varepsilon_{mk},$$

where

y_{mk} = grain mold rating of a particular line;

μ = overall mean;

Q_m = response due to m^{th} allele;

R_k = response due to k^{th} replication;

and ε_{mk} = random error associated with a particular observation.

Test of significance were based on a mixed model where the two homozygous QTL classes were treated as fixed effects, and replications were treated as random effects (Table 7).

Table 7. Expected mean squares for analysis of variance, each parent x environment analyzed separately.

Source	EMS
QTL	$\sigma_e^2 + r\delta_Q^2$
Replications(Locations)	$\sigma_e^2 + q\sigma_R^2$
Error	σ_e^2

The rationale behind these analyses was to determine whether significant variation existed within each of the listed sources of variation. The detection of statistically significant effects for a given source of variation indicates that differences exist among the classes which comprise that source. If no significant variation is indicated by the test, then it is assumed that no differences exist among the classes within that particular source of variation. If differences are detected, mean separation can then be performed to determine which of the treatments (parents, environments, and QTL, in this case) were statistically different.

In this study, the hypothesis is that selections carrying the allele from Sureño at the five grain mold QTL should have lower grain mold ratings, indicating that the

presence of this allele is improving grain mold resistance to a greater extent than even field based selection will allow. If this occurs, then it implies that MAS based on these QTLs will enhance grain mold resistance in sorghum breeding programs.

RESULTS

I. Field Evaluation

The grain mold rated at all locations was primarily grain weathering that occurred after physiological maturity. Significant variation among the parental lines was detected for grain mold rating (Tables 8 and 9). The parent*environment interactions were not statistically significant, indicating that the parents were consistent in their reaction across environments (Table 8). As expected, Sureño was the most resistant to grain mold, while RTx430 was the most susceptible (Table 9).

Table 8. Analysis of variance for grain mold, parental lines only.

Source		Sum of Squares	df	Mean Square	F	Sig.
Parents		88.634	5	17.727	20.557	.000
	Error	28.318	32.839	.862		
Locations		180.168	7	25.738	31.494	.000
	Error	10.640	13.019	.817		
Parents * Location		27.866	32	.871	1.761	.054
	Error	16.817	34	.495		
Reps(Location)		3.183	7	.455	.919	.504
	Error	16.817	34	.495		

Table 9. Mean separation of parental lines per se by Duncan's Least Significant Difference on basis of grain mold across eight environments in Texas in 2002.

Parent	Mean Grain Mold Rating*
Sureño	2.87[a]
RTx436	4.80[b]
BTx635	4.86[b]
Tx2903	4.93[b]
BTx631	5.07[b]
RTx430	6.43[c]

*Means within columns followed by the same letter do not differ ($P > 0.05$) as determined by ANOVA and Fisher's least significant difference.

As expected, significant differences were detected among environments for grain mold (Table 10). Grain mold damage ratings varied widely among environments (Fig. 1, Tables 10 and 11), ranging from a mean of 2.937 in Beaumont (least grain mold pressure) to 7.345 in Beeville, where the most disease pressure was encountered (grain mold scores of parental lines and checks were excluded from these analyses). Comparisons of the mold scores within all environments revealed that the eight respective environments effectively formed four groups of environments with mean grain mold scores which were statistically different (Fig. 1, Table 11). Although these environments clearly formed these four distinct groups, a Levene's test of homogeneity revealed that error variances for grain mold scores across the environments were not equal. Based on the heterogeneity and visual observations, it was deemed most

appropriate not to group the environments according to their apparent similarity in terms of grain mold rating, and all subsequent analyses were conducted treating these environments individually.

Table 10. Analysis of variance for grain mold in 87 F_5 progeny grown in eight environments across Texas in 2002.

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Parents	Hypothesis	100.143	4	25.036	7.798	.000
	Error	237.237	73.894	3.211		
Genotypes (Parent)	Hypothesis	225.968	82	2.756	3.864	.000
	Error	826.570	1159	.713		
Locations	Hypothesis	2297.923	7	328.275	190.943	.000
	Error	23.842	13.868	1.719		
Reps(Location)	Hypothesis	9.645	8	1.206	1.690	.096
	Error	826.570	1159	.713		
Parent*Location	Hypothesis	36.091	28	1.289	1.807	.006
	Error	826.570	1159	.713		

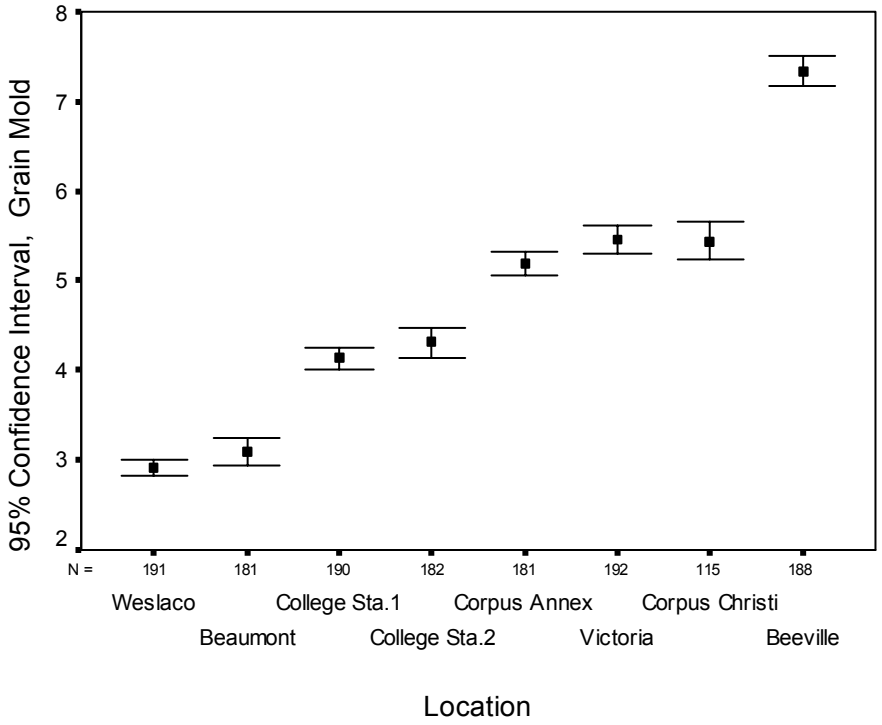

Figure 1. 95% Confidence intervals for grain mold ratings of all 87 F_5 lines, by environment.

Table 11. Mean grain mold ratings of all 87 F_5 lines, by environment.

Environment	Mean Grain Mold Rating*
Beeville	7.345[a]
Corpus Christi (Experiment Station)	5.459[b]
Victoria	5.339[b]
Corpus Christi (Station Annex)	5.230[b]
College Station (North)	4.302[c]
College Station (South)	4.126[c]
Beaumont	3.047[d]
Weslaco	2.937[d]

*Means within columns followed by the same letter do not differ ($P > 0.05$) as determined by ANOVA and Fisher's least significant difference.

There were significant correlations between the grain mold ratings of the lines across environments, although the correlation coefficients were not strikingly large in most cases (Table 12). As expected, both the two trials in College Station and the two trials in Corpus Christi were strongly correlated (.463 and .590, respectively). However, there was an even larger correlation between one of the two trials in College Station and the trial in Beaumont (.528) than there was between the two College Station trials. The grain mold ratings at Beeville were also strongly correlated to the two trials in Corpus Christi (.474 and .397).

In analyzing the F_5 progeny of the five adapted parents, significant differences were found among adapted parents, among genotypes within the adapted parents, and among environments (Table 10). Significant family*environment interactions were also detected, indicating that the progeny of a given parent did not necessarily perform the same across environments. Mean separation of the progeny by Duncan's Least Significant Difference according to their respective parental lines (Table 13) reveals that the populations derived from the adapted parents could be divided into three statistically different categories, with BTx635 yielding lines most resistant to grain mold, and Tx2903 giving rise to the least resistant lines. The progeny of BTx631, RTx436, and RTx430 lay between these two extremes, and were not statistically different from each other.

Table 12. Pearson correlation coefficients between mean grain mold ratings of all 87 F_5 lines at each environment.

	WES	BEA	CS1	CS2	CA	VIC	CC	BV
Weslaco (WES)	1.00	.391**	.275*	.091	.317**	.240*	.372**	.354**
Beaumont (BEA)	-	1.00	.528**	.320**	.315**	.366**	.349**	.143
College Station North (CS1)	-	-	1.00	.463**	.382**	.373**	.426**	.241*
College Station South (CS2)	-	-	-	1.00	.230*	.324**	.160	.219*
Corpus Christi Annex (CA)	-	-	-	-	1.00	.384**	.590**	.474**
Victoria (VIC)	-	-	-	-	-	1.00	.302**	.363**
Corpus Christi Station (CC)	-	-	-	-	-	-	1.00	.397**
Beeville (BV)	-	-	-	-	-	-	-	1.00

** Correlation is significant at the 0.01 level (2-tailed).
** Correlation is significant at the 0.05 level (2-tailed).

Table 13. Mean separation of adapted parents on basis of grain mold ratings of F_5 progeny.

Parent	Mean Grain Mold Score of progeny*
BTx635	4.19[a]
RTx436	4.63[b]
BTx631	4.74[b]
RTx430	4.76[b]
Tx2903	5.05[c]

*Means within columns followed by the same letter do not differ ($P > 0.05$) as determined by ANOVA and Fisher's least significant difference.

Days to mid-anthesis was correlated with the grain mold rating. Although the r^2 value was relatively low (.0501), there was nevertheless a noticeable trend toward later anthesis being positively correlated with increased grain mold (Fig. 2). This correlation was much more pronounced when environments were analyzed separately (Table 14) (Days to mid-anthesis were not recorded from either the Victoria or College Station South locations). In general, later maturity was associated with higher grain mold scores in environments with moderate grain mold pressure (i.e., the correlation coefficients were larger in these environments). This is opposite of the typical relationship between

grain mold and anthesis. The presence of the current relationship actually indicates that in most environments, grain molding conditions were not encountered until after all lines had reached physiological maturity, effectively minimizing any real effect due to maturity and the onset of significant grain mold pressure.

As with days to mid-anthesis and grain mold rating, a relationship between grain mold rating and plant height was also detected (Table 14 and Fig. 3). In general, the strongest correlations existed in environments with moderate levels of grain mold pressure. While not always significant, the negative relationship between grain mold rating and plant height was detected in all environments. This means that reduced grain mold ratings were associated with taller plants (Fig. 3). This association has been reported in previous studies and is primarily seen in populations in which significant variation in height occurs, resulting in micro climates that are different at the different heights.

Table 14. Pearson correlation coefficients for grain mold (GM), height (HT), and days to mid-anthesis (DY), by environment.

LOC		GM	HT	DY
Beaumont	GM	1.000	-.149	-.304**
	HT		1.000	-.053
	DY			1.000
Beeville	GM	1.000	-.192*	-.035
	HT		1.000	.108
	DY			1.000
Corpus Christi Annex	GM	1.000	-.222**	-.172*
	HT		1.000	.142
	DY			1.000
Corpus Christi Station	GM	1.000	-.154	.014
	HT		1.000	.244*
	DY			1.000
College Station North	GM	1.000	-.326**	-.604**
	HT		1.000	.204**
	DY			1.000
College Station South	GM	1.000	-.428**	.
	HT		1.000	.
	DY			.
Victoria	GM	1.000	-.349**	.
	HT		1.000	.
				.
Weslaco	GM	1.000	-.025	-.171*
	HT		1.000	-.021
	DY			1.000

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

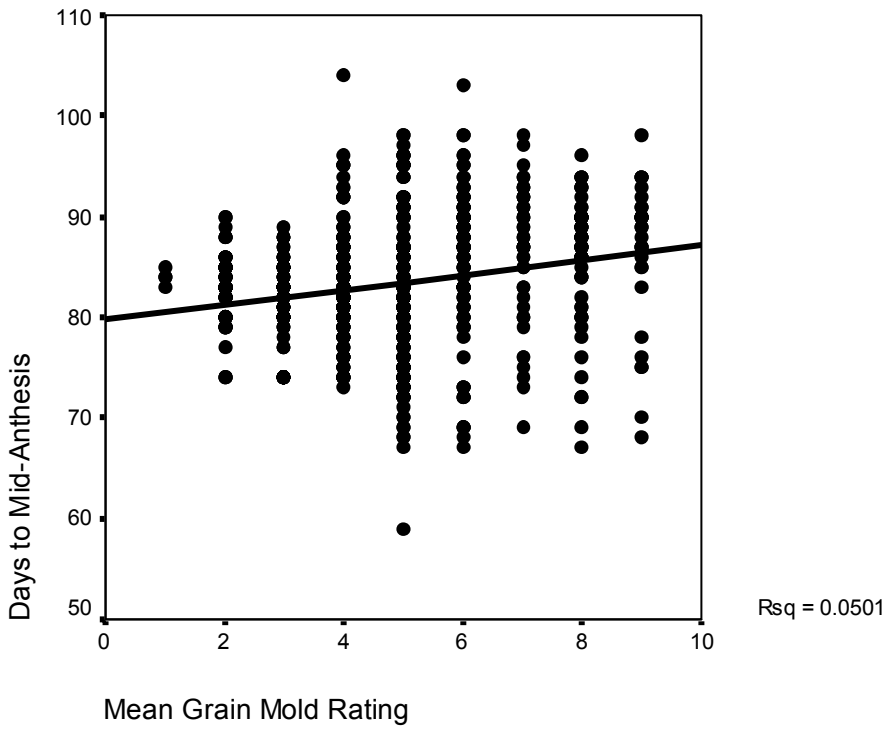

Figure 2. Correlation of days to mid-anthesis to grain mold rating for 87 F_5 lines grown in eight environments in Texas in 2002.

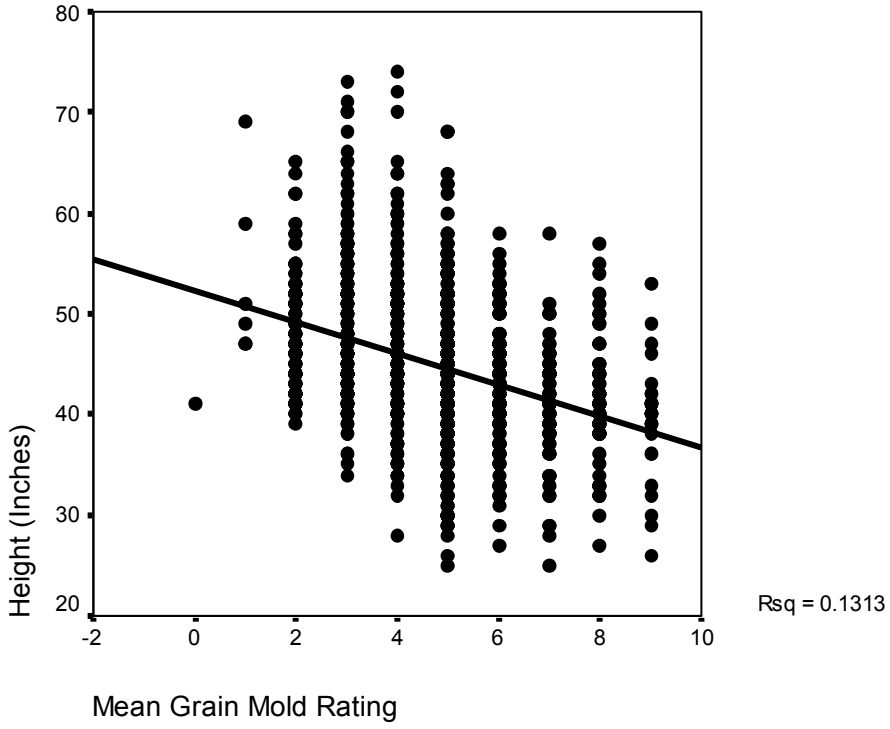

Figure 3. Correlation of height to grain mold rating for 87 F_5 lines grown in eight environment across Texas in 2002.

II. Molecular Marker Analysis

Since the lines in this study were selected based on desirable agronomic traits (maturity, height, etc.) in earlier generations, the populations used in this study were not of a suitable structure for the construction of a linkage map. Even though this was not a mapping study, there was good overall agreement between published linkage distances among markers and their observed segregation patterns within these populations. Those markers reported as tightly linked to each other tended to cosegregate, while those more loosely linked showed greater recombination frequencies.

In an F_4 population, genetic theory indicates that 87.5% of all loci should be homozygous (and split evenly between the two possible classes of homozygotes), and the remaining 12.5% of the loci should be heterozygous. This theory is contingent upon there being no artificial or natural selection, outcrossing, genetic drift, or mutations within the population under investigation. In the lines evaluated in this study, there was generally good agreement between expected and observed segregation ratios (Table 15). However, markers within several linkage groups did not segregate according to expectations. On linkage group D, the allele from the adapted parent using Xtxp177was present in a larger portion of the progeny than predicted by genetic segregation theory. On linkage group F, the Xtxp230 allele from the adapted parent was also more prevalent than would be expected from genetic theory. On linkage group I, the allele from Sureño with marker Xtxp57 was observed in a larger percentage of the progeny than should have been present. All markers from linkage group E showed significantly distorted segregation ratios, in favor of the adapted parent (Table 15).

Table 15. Chi-Square analysis of segregation ratios, codominant markers only. Expected segregation ratios were 7:2:7.

Marker	Linkage Group	Chi-Square Value	P
Xxtp12	D	.335	.846
Xtxp343	D	.989	.610
Xtxp177	D	6.690	.035*
Xtxa2943	E	8.590	.014*
Xtxp295	E	18.095	.000**
Xtxp168	E	6.186	.045*
Xtxp10	F	4.992	.082
Xtxp230	F	6.709	.035*
Xxtp67	F	.910	.634
Xxtp258	F	.369	.831
Xtxp287	F	.002	.999
Xtxp309	G	3.303	.192
Xtxp331	G	.214	.898
Xtxp217	G	2.156	.340
Xtxp270	G	1.355	.508
Xtxp130	G	1.526	.466
Xtxp274	I	3.398	.183
Xtxp95	I	2.504	.286
Xxtp57	I	6.370	.041*
Xtxa2549	I	4.914	.086

As expected, the five adapted parents varied widely with respect to the proportion of the molecular markers which were polymorphic between them and Sureño (Table 16). BTx631 showed the greatest amount of polymorphism, while BTx635 showed the least.

Table 16. Proportion of total markers polymorphic, by population.

Population	Proportion of markers polymorphic
BTx631	.845
RTx430	.812
RTx436	.812
Tx2903	.606
BTx635	.333

III. QTL Effects on Phenotypic Grain Mold Ratings

When the effects of each QTL were examined across all populations and environments (excluding the parental effects), only the Sureño alleles in the QTL on linkage group F enhanced the level of grain mold resistance (Tables 17-22). For the remaining four QTL, there was no difference in the grain mold rating of the lines carrying the adapted allele from those carrying the Sureño allele.

Table 17. Analysis of variance for QTL effects across all environments and populations, linkage group D.

Tests of Between-Subjects Effects, Linkage Group D QTL

Dependent Variable: GM

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	15125.345	1	15125.345	84.707	.000
	Error	1250.222	7.002	178.562		
Locations	Hypothesis	1274.786	7	182.112	192.707	.002
	Error	2.381	2.520	.945		
Reps(Locations)	Hypothesis	13.302	8	1.663	1.579	.128
	Error	711.911	676	1.053		
Allele	Hypothesis	.640	1	.640	1.820	.215
	Error	2.722	7.737	.352		
Location*Allele	Hypothesis	2.381	7	.340	.323	.944
	Error	711.911	676	1.053		

Table 18. Analysis of variance for QTL effects across all environments and populations, linkage group E.

Tests of Between-Subjects Effects, Linkage Group E QTL

Dependent Variable: GM

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	19885.872	1	19885.872	80.667	.000
	Error	1725.926	7.001	246.517		
Location	Hypothesis	1767.608	7	252.515	158.327	.000
	Error	9.410	5.900	1.595		
Reps(Location)	Hypothesis	7.148	8	.893	.983	.448
	Error	1025.318	1128	.909		
Allele	Hypothesis	9.751E-03	1	9.751E-03	.006	.940
	Error	11.431	7.157	1.597		
Location*Allele	Hypothesis	11.275	7	1.611	1.772	.089
	Error	1025.318	1128	.909		

Table 19. Analysis of variance for QTL effects across all environments and populations, linkage group F.

Tests of Between-Subjects Effects, Linkage Group F QTL

Dependent Variable: GM

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	11929.735	1	11929.735	89.664	.000
	Error	931.516	7.001	133.050		
Location	Hypothesis	943.268	7	134.753	119.242	.000
	Error	4.422	3.913	1.130		
Reps(Location)	Hypothesis	5.661	8	.708	.755	.643
	Error	485.809	518	.938		
Allele	Hypothesis	11.537	1	11.537	8.507	.022
	Error	9.637	7.106	1.356		
Location*Allele	Hypothesis	9.525	7	1.361	1.451	.183
	Error	485.809	518	.938		

Table 20. Analysis of variance for QTL effects across all environments and populations, linkage group G.

Tests of Between-Subjects Effects, Linkage Group G

Dependent Variable: GM

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	21760.849	1	21760.849	79.694	.000
	Error	1911.708	7.001	273.056		
Location	Hypothesis	1956.037	7	279.434	314.211	.000
	Error	2.982	3.354	.889		
Rep(Location)	Hypothesis	8.477	8	1.060	1.066	.385
	Error	979.999	986	.994		
Allele	Hypothesis	2.480	1	2.480	3.004	.125
	Error	6.035	7.308	.826		
Location*Allele	Hypothesis	5.759	7	.823	.828	.564
	Error	979.999	986	.994		

Table 21. Analysis of variance for QTL effects across all environments and populations, linkage group I.

Tests of Between-Subjects Effects, Linkage Group I

Dependent Variable: GM

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	11342.963	1	11342.963	81.690	.000
	Error	972.376	7.003	138.854		
Location	Hypothesis	996.878	7	142.411	2355.078	.785
	Error	2.619E-03	4.331E-02	6.047E-02		
Rep(Location)	Hypothesis	6.777	8	.847	.750	.647
	Error	591.683	524	1.129		
Allele	Hypothesis	1.269	1	1.269	3.829	.084
	Error	2.824	8.519	.331		
Location*Allele	Hypothesis	2.162	7	.309	.274	.964
	Error	591.683	524	1.129		

Table 22. Mean effects of each allele (adapted or Sureño) at each QTL on grain mold response across all populations and environments, by parental source of QTL.

QTL (by Linkage Group)	Adapted	Sureño
D	4.68	4.73^{ns}
E	4.68	4.71^{ns}
F	4.88	4.57*
G	4.75	4.64^{ns}
I	4.95	4.85^{ns}

When analyzed by environment, the effect of each QTL was not necessarily consistent across environments (Tables 23-27, Figures 4-8). The QTL located on linkage group D was found to be ineffective in reducing grain mold ratings across all environments (Table 23, Figure 4). Although the QTL on linkage group E was not effective when examined across all environments, it was nevertheless associated with a significant reduction in grain mold ratings in both Beaumont and one of the two College Station trials (Table 24, Figure 5). The linkage group F QTL, although the only QTL found to be significant across all environments, was only associated with a statistically significant reduction in one of the two Corpus Christi trials (Table 25, Figure 6). However, it can be seen that this QTL was associated with reductions in grain mold ratings in almost every environment. The QTL on linkage group G was found to only be effective in a single environment (Beaumont) in reducing grain mold incidence (Table 26, Figure 7), and the linkage group I QTL was not found to be statistically effective in any individual environment (Table 27, Figure 8).

Table 23. Mean effects of linkage group D QTL on grain mold rating, by environment, based on 87 F_5 lines from five different populations.

	Allelic Source		
Location	Adapted	Sureño	Difference
Weslaco	2.86	3.00	0.14^{ns}
Beaumont	3.08	3.00	-0.08^{ns}
College Station (South)	3.98	4.26	0.28^{ns}
College Station (North)	4.34	4.32	-0.02^{ns}
Corpus Christi (Annex)	5.24	5.29	0.05^{ns}
Corpus Christi (Station)	5.50	5.69	0.19^{ns}
Victoria	5.42	5.38	-0.04^{ns}
Beeville	7.25	7.23	-0.02^{ns}

*, ** indicate means in the row are statistically different from each other at $P < 0.05$ and 0.01, respectively.

Table 24. Mean effects of linkage group E QTL on grain mold rating, by environment, based on 87 F_5 lines from five different populations.

	Allelic Source		
Location	Adapted	Sureño	Difference
Weslaco	2.99	2.91	-0.08^{ns}
Beaumont	3.15	2.85	-0.3*
College Station (South)	4.07	4.38	0.3^{ns}
College Station (North)	4.38	4.00	-0.38**
Corpus Christi (Annex)	5.12	5.27	0.15^{ns}
Corpus Christi (Station)	5.43	5.50	0.07^{ns}
Victoria	5.33	5.36	0.03^{ns}
Beeville	7.28	7.50	0.22^{ns}

*, ** indicate means in the row are statistically different other at $P < 0.05$ and 0.01, respectively.

Table 25. Mean effects of linkage group F QTL on grain mold rating, by environment, based on 87 F_5 lines from five different populations.

	Allelic Source		
Location	Adapted	Sureño	Difference
Weslaco	3.03	2.85	-0.18^{ns}
Beaumont	3.06	3.09	0.03^{ns}
College Station (South)	4.32	4.32	0^{ns}
College Station (North)	4.41	4.24	-0.17^{ns}
Corpus Christi (Annex)	5.60	4.84	-0.76**
Corpus Christi (Station)	5.79	5.13	-0.66^{ns}
Victoria	5.53	5.32	-0.21^{ns}
Beeville	7.42	6.97	-0.45^{ns}

*, ** indicate means in the row are statistically different at $P < 0.05$ and 0.01, respectively.

Table 26. Mean effects of linkage group G QTL on grain mold rating, by environment, based on 87 F_5 lines from five different populations.

	Allelic Source		
Location	Adapted	Sureño	Difference
Weslaco	2.91	2.94	0.03^{ns}
Beaumont	3.22	2.86	-0.36*
College Station (South)	4.18	4.01	-0.17^{ns}
College Station (North)	4.37	4.08	-0.29^{ns}
Corpus Christi (Annex)	5.23	5.18	-0.05^{ns}
Corpus Christi (Station)	5.43	5.59	0.16^{ns}
Victoria	5.41	5.34	-0.07^{ns}
Beeville	7.38	7.34	-0.04^{ns}

*, ** indicate means in the row are statistically different from at $P < 0.05$ and 0.01, respectively.

Table 27. Mean effects of linkage group I QTL on grain mold rating, by environment, based on 87 F_5 lines from five different populations.

	Allelic Source		
Location	Adapted	Sureño	Difference
Weslaco	3.08	3.00	-0.08^{ns}
Beaumont	3.38	3.06	-0.32^{ns}
College Station (South)	4.29	4.32	0.03^{ns}
College Station (North)	4.38	4.44	0.06^{ns}
Corpus Christi (Annex)	5.50	5.45	-0.05^{ns}
Corpus Christi (Station)	5.87	5.75	-0.12^{ns}
Victoria	5.58	5.54	-0.04^{ns}
Beeville	7.87	7.55	-0.32^{ns}

*, ** indicate means in the row are statistically different at $P < 0.05$ and 0.01, respectively.

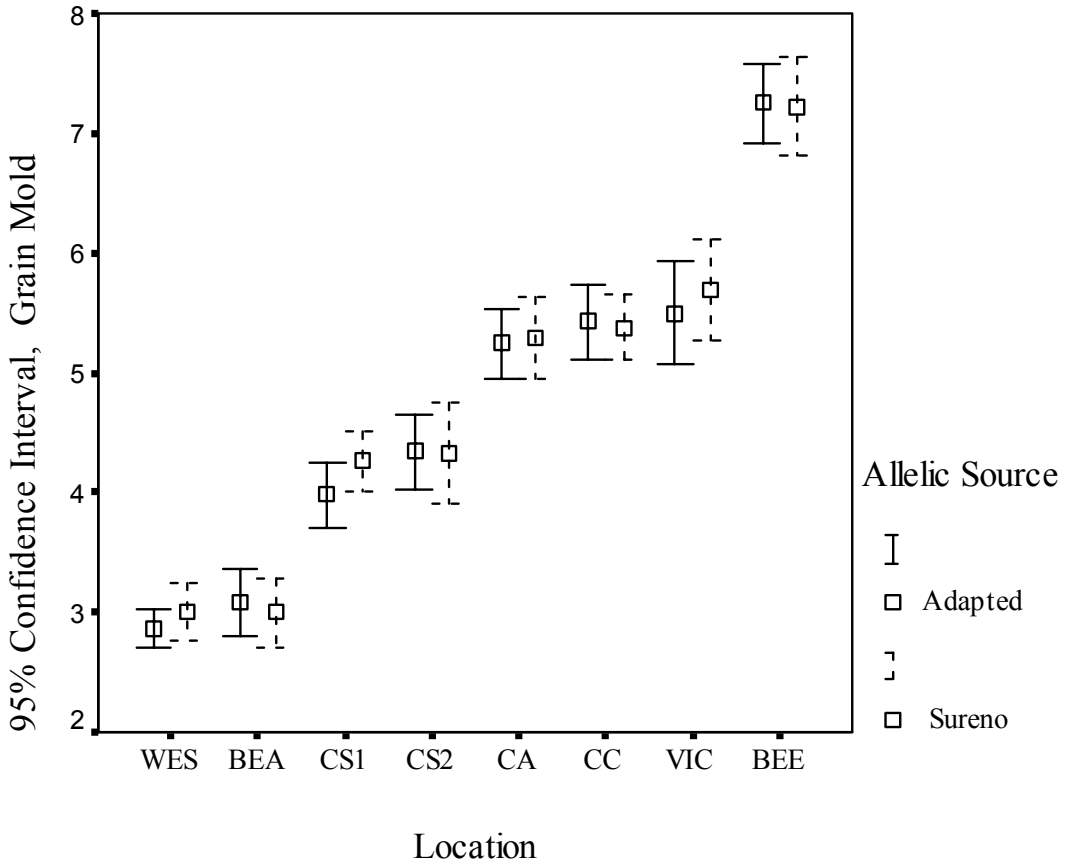

Figure 4. 95% Confidence intervals for grain mold ratings, linkage group D for 87 F_5 lines grown in eight environments in Texas. Abbreviations for the environment are: WES – Weslaco, BEA – Beaumont, CS1 – College Station North, CS2 – College Station South, CA – Corpus Christi Annex, CC – Corpus Christi, VIC – Victoria, and BEE – Beeville.

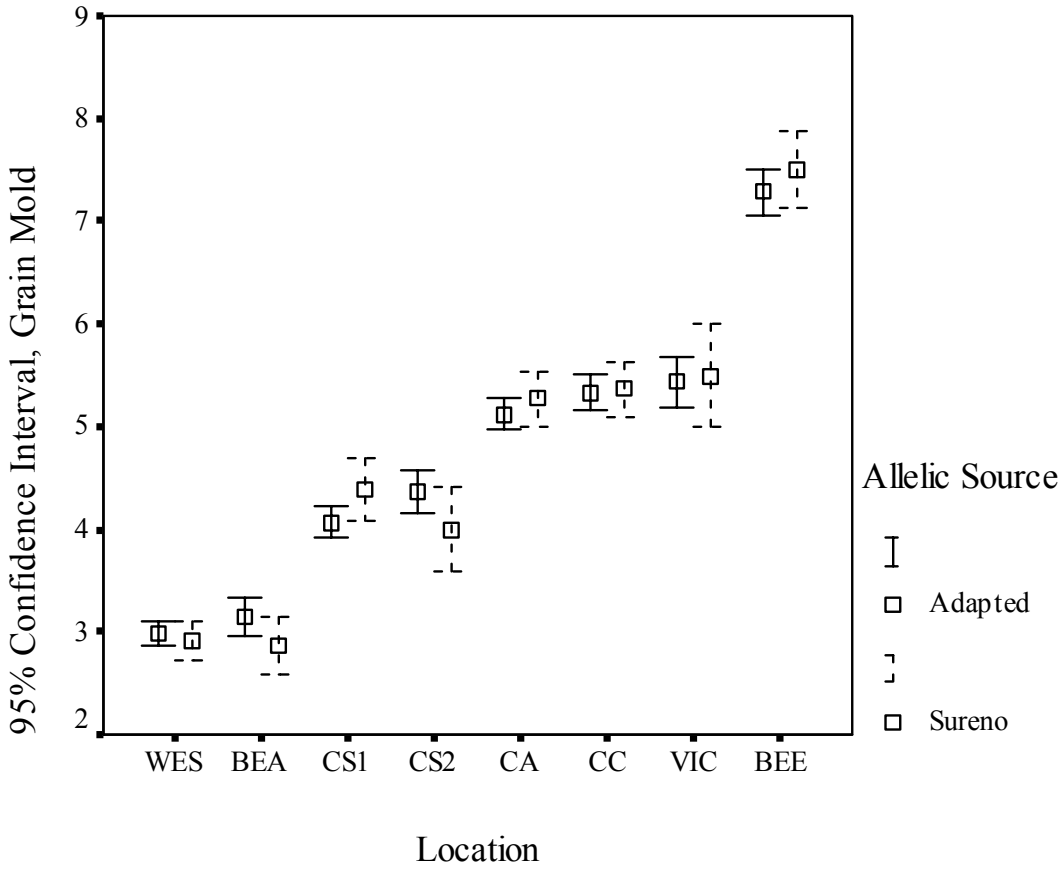

Figure 5. 95% Confidence intervals for grain mold Ratings, linkage group E for 87 F_5 lines grown in eight environments in Texas. Abbreviations for the environment are: WES – Weslaco, BEA – Beaumont, CS1 – College Station North, CS2 – College Station South, CA – Corpus Christi Annex, CC – Corpus Christi, VIC – Victoria, and BEE – Beeville.

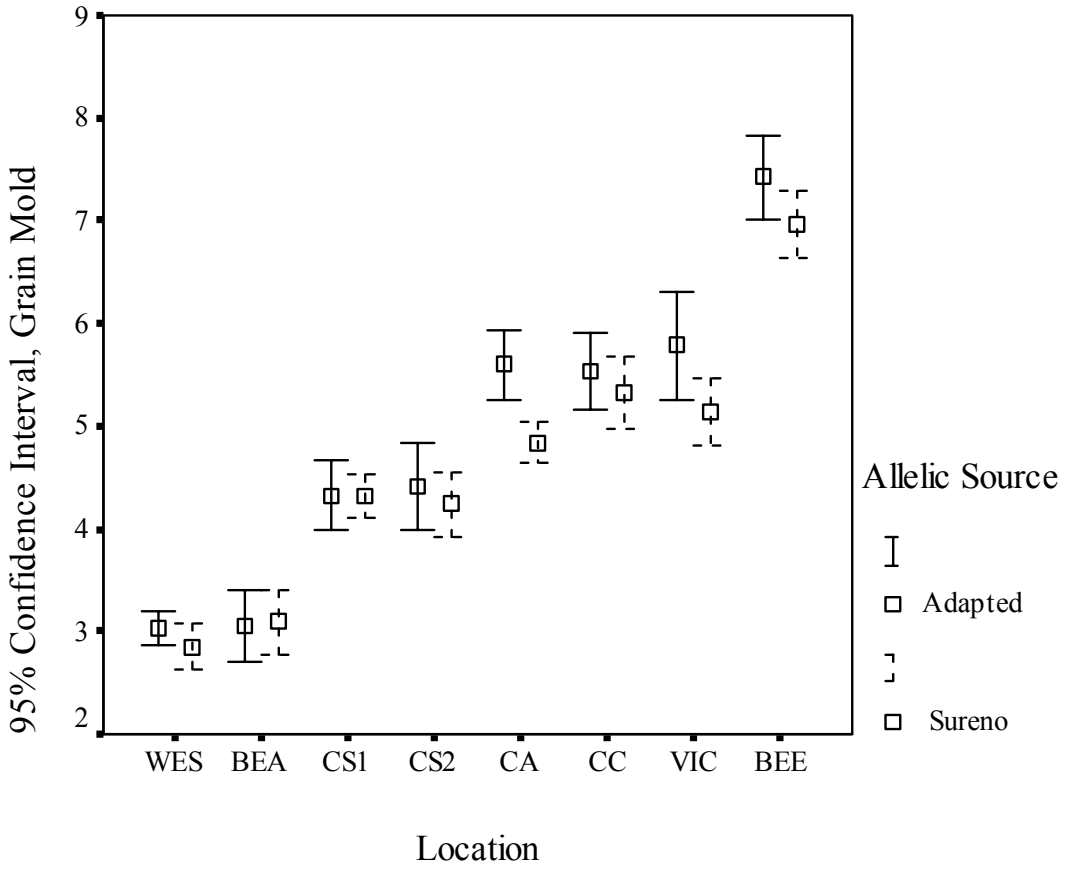

Figure 6. 95% Confidence intervals for grain mold Ratings, linkage group F for 87 F_5 lines grown in eight environments in Texas. Abbreviations for the environment are: WES – Weslaco, BEA – Beaumont, CS1 – College Station North, CS2 – College Station South, CA – Corpus Christi Annex, CC – Corpus Christi, VIC – Victoria, and BEE – Beeville. .

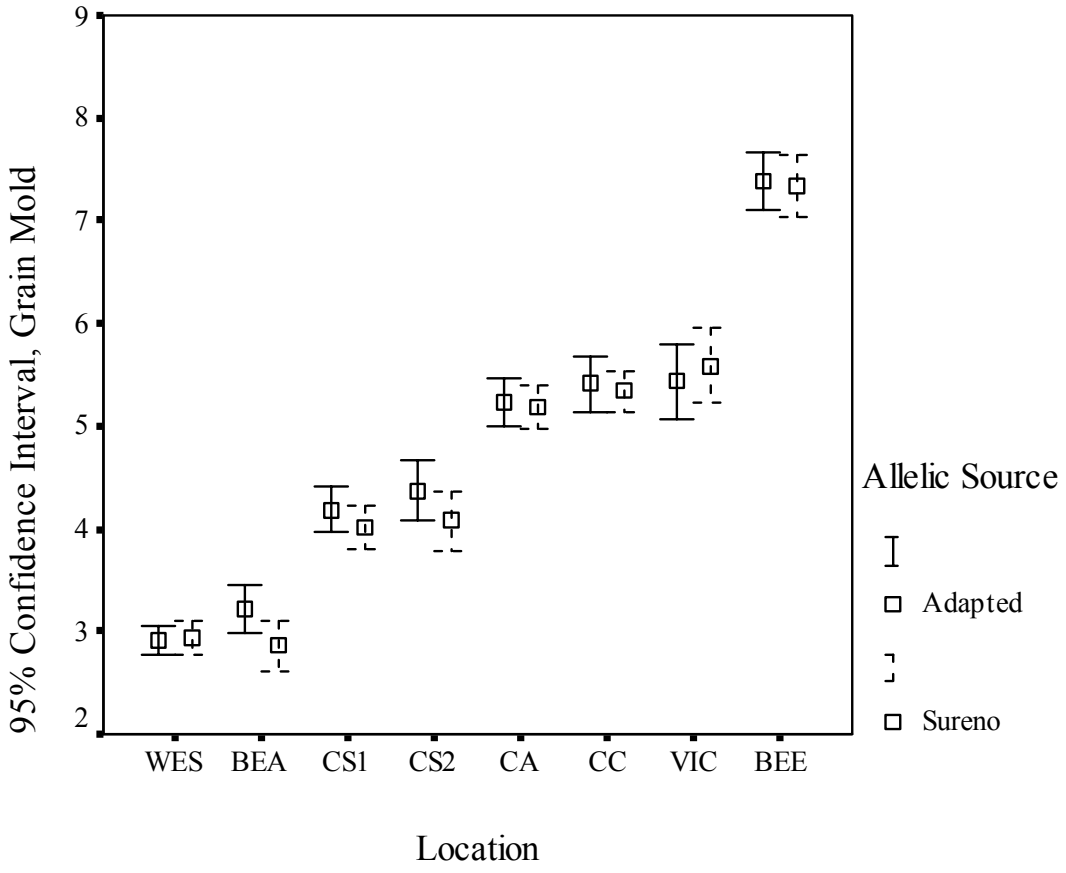

Figure 7. 95% Confidence intervals for grain mold Ratings, linkage group G for 87 F_5 lines grown in eight environments in Texas. Abbreviations for the environment are: WES – Weslaco, BEA – Beaumont, CS1 – College Station North, CS2 – College Station South, CA – Corpus Christi Annex, CC – Corpus Christi, VIC – Victoria, and BEE – Beeville. .

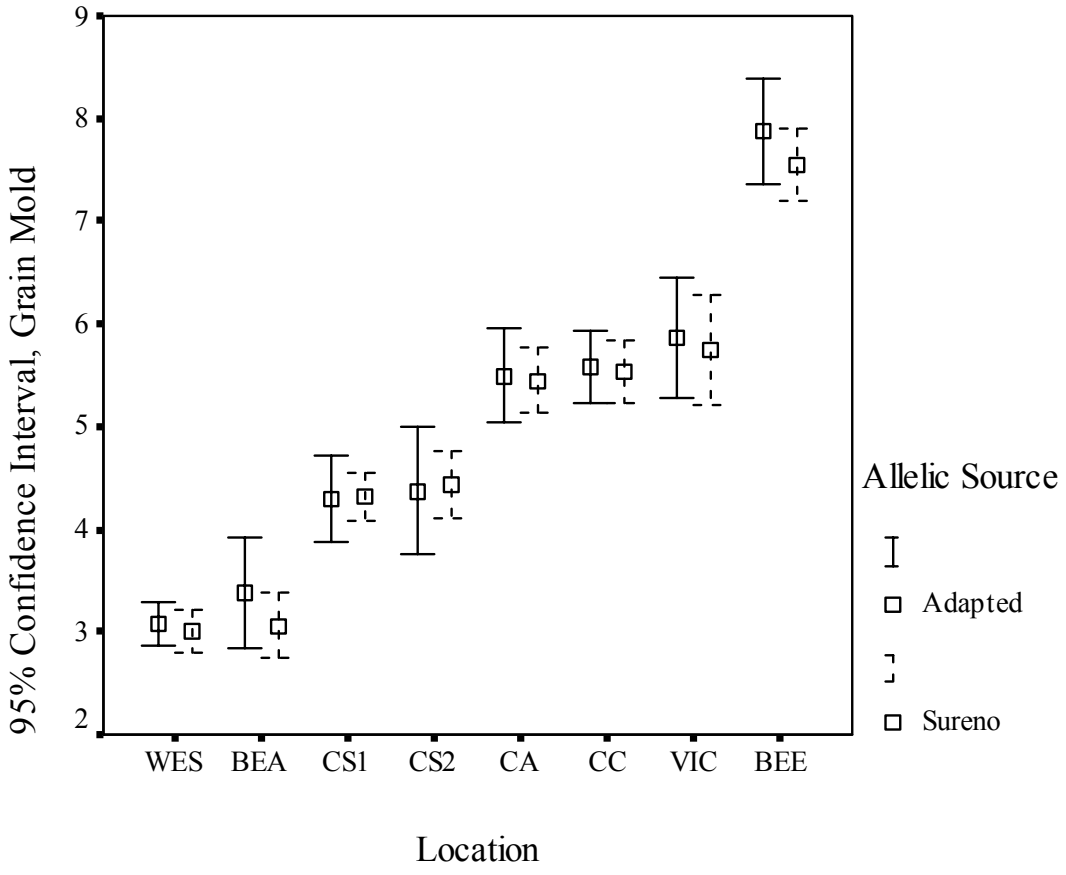

Figure 8. 95% Confidence intervals for grain mold Ratings, linkage group I for 87 F_5 lines grown in eight environments in Texas. Abbreviations for the environment are: WES – Weslaco, BEA – Beaumont, CS1 – College Station North, CS2 – College Station South, CA – Corpus Christi Annex, CC – Corpus Christi, VIC – Victoria, and BEE – Beeville.

The effects of the five QTL in each of the five populations were much more pronounced within the progeny of certain crosses (Tables 28-37). In lines derived from the RTx430/Sureño cross, there was a statistically significant reduction in grain mold ratings for lines which possessed the Sureño allele for all five QTL (Table 33) . For this population, there was a overall mean reduction in grain mold rating of 0.71with any given allele. The greatest reduction was associated with the QTL located on linkage group F, which reduced grain mold rating by an average of 1.05, and the least mean reduction was 0.49, associated with the linkage group G QTL.

Due to a lack of polymorphism, the QTL on linkage group I could not be analyzed exclusively within the RTx436/Sureño population. In this population, there was no statistically significant reduction associated with any of the other four QTL analyzed (Table 34). The mean effect of the presence of the Sureño allele across all QTL for this cross was a net increase in the grain mold rating of 0.04. In only one case (that of the linkage group E QTL) was there any reduction in the grain mold rating associated with the Sureño allele, and this reduction was very slight (-0.12).

There were no significant differences among any of the QTL allele classes within the BTx631/Sureño population (Table 35). The mean effect of the Sureño alleles, across all linkage groups, was an increase in .05 of the grain mold rating of those lines that inherited them.

Due to limited numbers of individuals in one allele class within the BTx635/Sureño population, it was not possible to analyze the effects of the QTL on linkage groups E or I. In linkage group E, an overabundance of the adapted allele class precluded the analysis, and for linkage group I, no suitable polymorphic markers were found that allowed scoring. There was a statistically significant difference among the two allelic classes for the QTL located on linkage group G (Table 36). In this case, the presence of the Sureño allele was associated with an increase in the grain mold score of 0.32. For the QTL on linkage groups D and F, there were virtually no detectable differences between the two allelic classes.

Within the Tx2903/Sureño population, statistically significant differences were detected among the two allelic classes for three of the five QTL. For the linkage group D QTL, there was a net increase of 0.14 associated with the presence of the Sureño allele (Table 37). There were statistically significant reductions in grain mold ratings associated with the QTL on linkage groups F and G of 0.44 and 0.31, respectively.

Table 28. Tests of between-subjects effects, linkage group D QTL, by population.

Parent	Source		Type III Sum of Squares	df	Mean Square	F	Sig.
RTx430	Location	Hypothesis	150.146	7	21.449	13.330	.005
		Error	8.499	5.282	1.609		
	Rep(Location)	Hypothesis	7.668	8	.958	1.000	.450
		Error	43.149	45	.959		
	Allele	Hypothesis	7.390	1	7.390	5.038	.050
		Error	14.053	9.580	1.467		
	Location*Allele	Hypothesis	11.301	7	1.614	1.684	.137
		Error	43.149	45	.959		
RTx436	Location	Hypothesis	148.811	7	21.259	21.241	.001
		Error	5.965	5.960	1.001		
	Rep(Location)	Hypothesis	4.271	8	.534	1.088	.380
		Error	40.740	83	.491		
	Allele	Hypothesis	.765	1	.765	.802	.400
		Error	6.746	7.068	.954		
	Location*Allele	Hypothesis	6.711	7	.959	1.953	.071
		Error	40.740	83	.491		
BTx631	Location	Hypothesis	177.293	7	25.328	23.661	.002
		Error	5.157	4.817	1.070		
	Rep(Location)	Hypothesis	4.084	8	.510	.809	.596
		Error	51.722	82	.631		
	Allele	Hypothesis	.192	1	.192	.163	.698
		Error	8.415	7.143	1.178		
	Location*Allele	Hypothesis	8.320	7	1.189	1.884	.083
		Error	51.722	82	.631		
BTx635	Location	Hypothesis	269.867	7	38.552	29.688	.001
		Error	6.250	4.813	1.299		
	Rep(Location)	Hypothesis	13.007	8	1.626	2.208	.030
		Error	103.805	141	.736		
	Allele	Hypothesis	1.144E-02	1	1.144E-02	.027	.874
		Error	3.199	7.526	.425		
	Location*Allele	Hypothesis	2.930	7	.419	.569	.780
		Error	103.805	141	.736		
Tx2903	Location	Hypothesis	534.269	7	76.324	86.508	.014
		Error	1.663	1.885	.882		
	Rep(Location)	Hypothesis	14.302	8	1.788	1.463	.172
		Error	279.832	229	1.222		
	Allele	Hypothesis	2.701	1	2.701	8.093	.021
		Error	2.739	8.206	.334		
	Location*Allele	Hypothesis	2.204	7	.315	.258	.969
		Error	279.832	229	1.222		

Table 29. Tests of between-subjects effects, linkage group E QTL, by population.

PARENT	Source		Type III Sum of Squares	df	Mean Square	F	Sig.
RTx430	Location	Hypothesis	380.890	7	54.413	53.975	.002
		Error	3.436	3.409	1.008		
	Rep(Location)	Hypothesis	9.353	8	1.169	1.054	.399
		Error	155.340	140	1.110		
	Allele	Hypothesis	8.943	1	8.943	9.319	.014
		Error	8.276	8.625	.960		
	Location*Allele	Hypothesis	6.619	7	.946	.852	.546
		Error	155.340	140	1.110		
RTx436	Location	Hypothesis	446.393	7	63.770	72.847	.000
		Error	4.564	5.213	.875		
	Rep(Location)	Hypothesis	6.451	8	.806	1.330	.229
		Error	159.440	263	.606		
	Allele	Hypothesis	1.373	1	1.373	2.032	.196
		Error	4.816	7.129	.676		
	Location*Allele	Hypothesis	4.734	7	.676	1.116	.353
		Error	159.440	263	.606		
BTx631	Location	Hypothesis	114.676	7	16.382	36.822	.000
		Error	2.292	5.153	.445		
	Rep(Location)	Hypothesis	2.887	8	.361	.585	.790
		Error	122.818	199	.617		
	Allele	Hypothesis	1.376	1	1.376	2.660	.138
		Error	4.624	8.939	.517		
	Location*Allele	Hypothesis	3.546	7	.507	.821	.571
		Error	122.818	199	.617		
BTx635	Location	Hypothesis	369.636	7	.	.	.
		Error	.	.	.		
	Rep(Location)	Hypothesis	5.140	8	.642	.856	.555
		Error	145.576	194	.750		
	Allele	Hypothesis	.000	0	.	.	.
		Error	.	.	.		
	Location*Allele	Hypothesis	.000	0	.	.	.
		Error	.	.	.		
Tx2903	Location	Hypothesis	505.555	7	72.222	50.939	.001
		Error	5.863	4.135	1.418		
	Rep(Location)	Hypothesis	6.221	8	.778	.688	.702
		Error	275.874	244	1.131		
	Allele	Hypothesis	2.765	1	2.765	1.602	.245
		Error	12.305	7.129	1.726		
	Location*Allele	Hypothesis	12.141	7	1.734	1.534	.156
		Error	275.874	244	1.131		

Table 30. Tests of between-subjects effects, linkage group F QTL, by population.

PARENT	Source		Type III Sum of Squares	df	Mean Square	F	Sig.
RTx430	Location	Hypothesis	85.641	7	12.234	11.396	.033
		Error	3.311	3.084	1.074		
	Rep(Location)	Hypothesis	3.548	8	.443	.473	.862
		Error	20.619	22	.937		
	Allele	Hypothesis	10.714	1	10.714	7.240	.029
		Error	10.995	7.429	1.480		
	Location*Allele	Hypothesis	10.545	7	1.506	1.607	.186
		Error	20.619	22	.937		
RTx436	Location	Hypothesis	205.741	7	29.392	50.907	.008
		Error	1.466	2.539	.577		
	Rep(Location)	Hypothesis	7.797	8	.975	1.466	.177
		Error	75.106	113	.665		
	Allele	Hypothesis	9.946E-02	1	9.946E-02	.359	.567
		Error	2.069	7.464	.277		
	Location*Allele	Hypothesis	1.904	7	.272	.409	.895
		Error	75.106	113	.665		
BTx631	Location	Hypothesis	103.136	7	14.734	48.154	.094
		Error	.334	1.091	.306		
	Rep(Location)	Hypothesis	1.169	8	.146	.228	.985
		Error	43.631	68	.642		
	Allele	Hypothesis	.294	1	.294	.394	.550
		Error	5.341	7.151	.747		
	Location*Allele	Hypothesis	5.237	7	.748	1.166	.334
		Error	43.631	68	.642		
BTx635	Location	Hypothesis	162.639	7	23.234	33.309	.005
		Error	2.372	3.400	.698		
	Rep(Location)	Hypothesis	5.303	8	.663	.906	.519
		Error	38.064	52	.732		
	Allele	Hypothesis	1.627E-03	1	1.627E-03	.002	.964
		Error	5.413	7.082	.764		
	Location*Allele	Hypothesis	5.352	7	.765	1.044	.412
		Error	38.064	52	.732		
Tx2903	Location	Hypothesis	316.007	7	45.144	42.435	.002
		Error	4.057	3.813	1.064		
	Rep(Location)	Hypothesis	7.968	8	.996	.855	.556
		Error	194.516	167	1.165		
	Allele	Hypothesis	7.235	1	7.235	6.003	.042
		Error	9.038	7.500	1.205		
	Location*Allele	Hypothesis	8.446	7	1.207	1.036	.408
		Error	194.516	167	1.165		

Table 31. Tests of between-subjects effects, linkage group G QTL, by population.

PARENT	Source		Type III Sum of Squares	df	Mean Square	F	Sig.
RTx430	Location	Hypothesis	266.627	7	38.090	36.728	.004
		Error	3.531	3.404	1.037		
	Rep(Location)	Hypothesis	14.140	8	1.768	1.501	.164
		Error	134.213	114	1.177		
	Allele	Hypothesis	5.075	1	5.075	8.928	.015
		Error	5.089	8.954	.568		
	Location*Allele	Hypothesis	3.725	7	.532	.452	.867
		Error	134.213	114	1.177		
RTx436	Location	Hypothesis	272.643	7	38.949	49.774	.001
		Error	3.405	4.352	.783		
	Rep(Location)	Hypothesis	6.939	8	.867	1.309	.242
		Error	105.346	159	.663		
	Allele	Hypothesis	.604	1	.604	1.037	.342
		Error	4.133	7.092	.583		
	Location*Allele	Hypothesis	4.077	7	.582	.879	.525
		Error	105.346	159	.663		
BTx631	Location	Hypothesis	406.777	7	58.111	97.636	.002
		Error	1.723	2.895	.595		
	Rep(Location)	Hypothesis	7.670	8	.959	1.612	.125
		Error	99.916	168	.595		
	Allele	Hypothesis	9.522E-02	1	9.522E-02	.381	.553
		Error	2.221	8.875	.250		
	Location*Allele	Hypothesis	1.632	7	.233	.392	.906
		Error	99.916	168	.595		
BTx635	Location	Hypothesis	366.971	7	52.424	37.833	.000
		Error	8.258	5.960	1.386		
	Rep(Location)	Hypothesis	6.044	8	.755	1.073	.384
		Error	142.237	202	.704		
	Allele	Hypothesis	7.629	1	7.629	5.729	.047
		Error	9.446	7.094	1.332		
	Location*Allele	Hypothesis	9.377	7	1.340	1.902	.071
		Error	142.237	202	.704		
Tx2903	Location	Hypothesis	603.906	7	86.272	72.798	.001
		Error	4.353	3.673	1.185		
	Rep(Location)	Hypothesis	11.758	8	1.470	1.247	.272
		Error	291.077	247	1.178		
	Allele	Hypothesis	7.463	1	7.463	8.292	.023
		Error	6.498	7.220	.900		
	Location*Allele	Hypothesis	6.277	7	.897	.761	.621
		Error	291.077	247	1.178		

Table 32. Tests of between-subjects effects, linkage group I QTL, by population.

PARENT	Source		Type III Sum of Squares	df	Mean Square	F	Sig.
RTx430	Location	Hypothesis	139.229	7	19.890	34.939	.016
		Error	1.372	2.409	.569		
	Rep(Location)	Hypothesis	6.844	8	.855	.842	.569
		Error	78.240	77	1.016		
	Allele	Hypothesis	17.246	1	17.246	25.250	.002
		Error	4.210	6.163	.683		
	Location*Allele	Hypothesis	4.080	6	.680	.669	.675
		Error	78.240	77	1.016		
BTx631	Location	Hypothesis	260.607	7	37.230	102.343	.004
		Error	.918	2.523	.364		
	Rep(Location)	Hypothesis	4.313	8	.539	.770	.630
		Error	118.339	169	.700		
	Allele	Hypothesis	3.006	1	3.006	6.137	.038
		Error	4.006	8.178	.490		
	Location*Allele	Hypothesis	3.348	7	.478	.683	.686
		Error	118.339	169	.700		
Tx2903	Location	Hypothesis	598.283	7	85.469	129.937	.043
		Error	.788	1.198	.658		
	Rep(Location)	Hypothesis	13.609	8	1.701	1.289	.250
		Error	304.759	231	1.319		
	Allele	Hypothesis	.922	1	.922	3.098	.118
		Error	2.306	7.753	.297		
	Location*Allele	Hypothesis	2.001	7	.286	.217	.981
		Error	304.759	231	1.319		

Table 33. Grain mold rating means and standard deviation of homozygous classes within RTx430/Sureño population, by parent.

Parent	Allele Source	Mean	Std. Deviation
D	Adapted	4.72*	1.97
	Sureño	4.10	1.61
E	Adapted	5.02*	1.76
	Sureño	4.48	1.94
F	Adapted	5.40*	2.03
	Sureño	4.35	1.38
G	Adapted	5.15*	1.86
	Sureño	4.66	1.81
I	Adapted	5.15**	1.67
	Sureño	4.31	1.66

*, ** indicate means in the row are statistically different at $P < 0.05$ and 0.01, respectively.

Table 34. Grain mold rating means and standard deviation of homozygous classes within RTx436/Sureño population, by linkage group.

Parent	Allele Source	Mean	Std. Deviation
D	Adapted	4.44	1.43
	Sureño	4.59	1.34
E	Adapted	4.68	1.41
	Sureño	4.56	1.54
F	Adapted	4.61	1.56
	Sureño	4.64	1.38
G	Adapted	4.57	1.47
	Sureño	4.66	1.48

*, ** indicate means in the row are statistically different at $P < 0.05$ and 0.01, respectively.

Table 35. Grain mold rating means and standard deviation of homozygous classes within population BTx631/Sureño, by linkage group.

Parent	Allele Source	Mean	Std. Deviation
D	Adapted	4.80	1.67
	Sureño	4.87	1.38
E	Adapted	4.80	1.54
	Sureño	4.64	1.78
F	Adapted	4.48	1.31
	Sureño	4.62	1.42
G	Adapted	4.77	1.62
	Sureño	4.71	1.67
I	Adapted	4.55*	1.61
	Sureño	4.83	1.57

*, ** indicate means in the row are statistically different at $P < 0.05$ and 0.01, respectively.

Table 36. Grain mold rating means and standard deviation of homozygous classes within population BTx635/Sureño, by linkage group.

Parent	Allele Source	Mean	Std. Deviation
D	Adapted	4.16	1.54
	Sureño	4.18	1.57
F	Adapted	4.32	1.81
	Sureño	4.31	1.64
G	Adapted	4.00*	1.42
	Sureño	4.32	1.66

*, ** indicate means in the row are statistically different at $P < 0.05$ and 0.01, respectively.

Table 37. Grain mold rating means and standard deviations of homozygous classes within population Tx2903/Sureño, by linkage group.

Parent	Allele Source	Mean	Std. Deviation
D	Adapted	5.08*	1.81
	Sureño	5.22	1.85
E	Adapted	5.52	2.03
	Sureño	4.91	1.77
F	Adapted	5.20*	1.89
	Sureño	4.76	1.57
G	Adapted	5.21*	1.81
	Sureño	4.91	1.91
I	Adapted	5.07	1.89
	Sureño	5.13	1.98

*, ** indicate means in the row are statistically different at $P < 0.05$ and 0.01, respectively.

Summaries of the analyses of the effects of each of the five QTL, by parent and environment, are shown in Tables 38-42. These summaries show the effects of a given QTL within each of the five populations, with the data from each of the eight environments analyzed separately. Although statistical significance was detected in several cases, the reduction in the size of the respective datasets when the classes were thus partitioned proportionately decreased the precision with which such differences could be detected. This division of the dataset also prevented the analysis of many of the QTL x environment groups.

The progeny of RTx430 (Table 38) showed overall reductions in grain mold ratings in 34 of the 39 QTL x environment combinations, and five of these reductions were statistically significant. When the effects of the five QTL within the RTx430/Sureño population were examined across all environments, statistically significant reductions were detected in all cases, indicating that the Sureño alleles consistently and effectively reduced grain mold incidence within this population and the markers were effective at improving grain mold resistance in the progeny. The greatest reductions within this population were associated with the QTL on linkage groups F and I, although substantial reductions were also associated with other three QTL.

The progeny of the RTx436/Sureño population showed much less consistent results for the four linkage groups that were available for analysis (Table 39). In only 14 of the 32 QTL x environment combinations was there a reduction in the grain mold rating, and only one case showed statistical significance (the linkage group E QTL in Beaumont). The combined analysis across environments did not indicate a statistically significant reduction in grain mold susceptibility for any linkage group.

Alleles from Sureño at the five QTL were not effective at reducing grain mold ratings in the lines from the BTx631/Sureño population (Table 40). In 21 of the 40 cases there were reductions in grain mold ratings, but none of these were statistically significant. The linkage group I QTL from Sureño was actually associated with a statistically significant increase in grain mold in one environment (College Station), and the mold scores were significantly higher when analyzed across all locations.

Overall lack of polymorphism within the BTx635/Sureño population prevented the analysis of the effects of the QTL on linkage groups E and I (Table 41). Of the 24 cases which could be analyzed, only eight proved to be associated with a net reduction in grain mold ratings for those progeny that possessed the Sureño allele. The QTL on linkage group F was the only case where a statistically significant reduction in grain mold was observed. Despite this reduction, the combined effect of the linkage group F was virtually nil when analyzed across all environments. The QTL on linkage group G was associated with an increase in grain mold ratings across all but one environment (College Station), and statistical significance was detected in two of these cases. In a combined analysis, the linkage group G Sureño allele was associated with statistically higher grain mold scores.

In the progeny derived from the cross of Tx2903/Sureño, results were variable with respect to the effects of the five QTL (Table 42). Across all environments, the QTL from linkage groups D, E, and I imparted a small increase in grain mold scores. This increase was statistically significant only for the linkage group D QTL. The linkage group F and G QTL were associated with a reduction in grain mold ratings in 13 of the 16 QTL x environment combinations, three of which were statistically significant. Moreover, both QTL were effective in reducing the grain mold ratings in the combined analysis across all locations, and statistical significance was detected in both of these analyses.

Table 38. Mean reduction in grain mold rating associated with presence of Sureño allele in progeny from the cross of RTx430/Sureño. A negative value indicates a reduction in grain mold rating due to the presence of the Sureño allele.

	Linkage Group				
Environment	D	E	F	G	I
Weslaco	-0.75	-0.17	-0.50	-0.14	-0.30
Beaumont	-0.75	-1.07*	+0.25	-0.16	-1.70*
College Sta. 1	+0.92	-0.08	-1.25	+0.15	-0.75
College Sta. 2	-0.40	-1.40	-2.00	-1.16	-1.33
Corpus Annex	-0.90	-0.37	-1.25*	-0.75*	-0.63
Victoria	-0.25	-0.58	-2.50*	-0.69	-0.70
Corpus Christi	-1.00	0	-0.77	0	-
Beeville	-1.92	-0.37	-0.20	-0.81	-1.43
Combined	-0.62*	-0.54**	-1.05**	-0.49*	-0.84**

*, ** indicate means in the row are statistically different at $P < 0.05$ and 0.01, respectively.

Table 39. Mean reduction in grain mold rating associated with presence of Sureño allele in progeny from the cross of RTx436/Sureño. A negative value indicates a reduction in grain mold rating due to the presence of the Sureño allele.

	Linkage Group				
Environment	D	E	F	G	I
Weslaco	+0.58	-0.16	+0.23	+0.28	-
Beaumont	+0.46	-0.53*	+0.38	-0.41	-
College Sta. 1	+0.30	+0.09	+0.16	+0.23	-
College Sta. 2	-1.08	-0.56	-0.05	+0.35	-
Corpus Annex	+0.25	+0.11	-0.34	+0.26	-
Victoria	+0.45	+0.12	-0.07	-0.39	-
Corpus Christi	+0.33	-0.14	+0.31	+0.25	-
Beeville	+0.08	-0.04	-0.20	+0.37	-
Combined	+0.15	-0.12	+0.03	+0.09	-

*, ** indicate means in the row are statistically different at $P < 0.05$ and 0.01, respectively.

Table 40. Mean reduction in grain mold rating associated with presence of Sureño allele in the progeny from the cross of BTx631/Sureño. A negative value indicates a reduction in grain mold rating due to the presence of the Sureño allele.

	Linkage Group				
Environment	D	E	F	G	I
Weslaco	+1.20	-0.11	0	+0.08	+0.15
Beaumont	-0.25	-1.11	0	-0.35	+0.36
College Sta. 1	-0.29	-0.54	+0.50	-0.12	+0.62
College Sta. 2	-0.24	-0.98	0	-0.21	+0.91*
Corpus Annex	-0.13	-0.36	-0.37	+0.29	+0.13
Victoria	-0.71	-0.32	+0.87	-0.13	+0.35
Corpus Christi	+0.52	+0.5	-0.80	-0.10	-0.12
Beeville	-0.05	+0.68	+0.75	+0.03	-0.15
Combined	+0.07	-0.16	+0.14	-0.06	+0.28*

*, ** indicate means in the row are statistically different at $P < 0.05$ and 0.01, respectively.

Table 41. Mean reduction in grain mold rating associated with presence of Sureño allele in the progeny from the cross of BTx635/Sureño. A negative value indicates a reduction in grain mold rating due to the presence of the Sureño allele.

	Linkage Group				
Environment	D	E	F	G	I
Weslaco	-0.27	-	-0.92*	+0.11	-
Beaumont	-0.15	-	+0.17	+0.05	-
College Sta. 1	+0.45	-	+0.58	+0.42	-
College Sta. 2	+0.03	-	+0.30	-0.39	-
Corpus Annex	-0.37	-	-0.27	+0.64*	-
Victoria	+0.05	-	+0.50	+0.97*	-
Corpus Christi	+0.43	-	+0.35	+0.80	-
Beeville	0	-	-0.75	+0.41	-
All	+0.02	-	-0.01	+0.32*	-

*, ** indicate means in the row are statistically different at $P < 0.05$ and 0.01, respectively.

Table 42. Mean reduction in grain mold rating associated with presence of Sureño allele in the progeny from the cross of Tx2903/Sureño. A negative value indicates a reduction in grain mold rating due to the presence of the Sureño allele.

	Linkage Group				
Environment	D	E	F	G	I
Weslaco	-0.02	-0.17	-0.06	+0.12	-0.14
Beaumont	-0.01	+0.19	-0.13	-0.73	-0.14
College Sta. 1	+0.19	+0.79*	-0.42	-0.80*	-0.01
College Sta. 2	+0.31	-0.60	+0.02	-0.30	+0.15
Corpus Annex	+0.31	+0.62	-0.93*	-0.50	+0.31
Victoria	-0.01	-0.75	-0.27	-0.36	+0.14
Corpus Christi	+0.37	+0.57	-1.58*	0	+0.58
Beeville	+0.53	+0.48	-0.10	-0.30	+0.18
Combined	+0.14*	+0.25	-0.44*	-0.30*	+0.06

*, ** indicate means in the row are statistically different at $P < 0.05$ and 0.01, respectively.

DISCUSSION AND CONCLUSION

The QTL used in this study were identified using a recombinant inbred line population derived from a cross between Sureño and RTx430 (Klein et al., 2001). In the current study, these QTL were very effective for MAS in the Sureño/RTx430 population. The findings presented here corroborate the existence and validity of these QTL, and demonstrate that they are applicable across a wide range of environments and levels of disease pressure. They also indicate that in this population MAS would be effective for grain mold resistance. However, the use of these markers in the remaining populations was not effective.

Therefore, these results illustrate a major limitation in the application of such technology in marker-assisted selection. Although certain QTL were also effective in a small number of cases (such as those in linkage groups F and G within the Tx2903/Sureño progeny), and in general, there was no advantage to possessing the Sureño allele.

An initial analysis of variance of the effects of each QTL across all environments and populations (Table 22) revealed a statistically significant (.05) reduction in the grain mold rating of lines possessing the Sureño allele for the QTL on linkage group F. When the effects of the QTL are examined separately by environment (Tables 33-37), it can be seen that the effects of most QTL are of varying magnitude and consistency on the grain mold rating. However, the QTL located on both linkage groups F and G show remarkable consistency in being associated with a net reduction in grain mold damage in

those lines which possess them. Although most of these cases are not statistically significant, there nevertheless appeared to be a small advantage in the Sureño alleles in terms of their ability to impart resistance to grain mold.

Analyzing the effects of the QTL within each of the five respective populations, however, showed that the vast preponderance of the observed benefits from these alleles were enjoyed by the progeny of only one cross. All five of the grain mold resistance QTL were associated with a statistically significant reduction (oftentimes of substantial magnitude) in the grain mold rating of those lines derived from the Sureño/RTx430 cross. The only other two statistically significant reductions in grain mold ratings associated with the Sureño allele was within the population Sureño/Tx2903 for the QTL located on linkage groups F and G. The three other cases which showed statistical significance (.05) were associated with a net increase in the grain mold ratings of those lines which contained the allele from Sureño.

The results of the field evaluation were as expected; there was a wide range in disease pressure across the eight environments in which the trial was evaluated. These environments provide a realistic glimpse of grain mold in a typical year, and supports the applicability of the findings presented here. Given the diversity of fungi that are associated with grain mold, and the genetic complexity of grain mold resistance, such an array of environments was necessary to encompass the breadth of disease pressure they might normally encounter. The differential responses of the grain mold resistance QTL across these various environments (Tables 23-27, Figures 4-8) point to the quantitative

nature of the trait itself; such environmental influences are a defining characteristic of quantitative traits.

It is perhaps intuitive that the time of evaluation for grain mold has significant ramifications in terms of the amount of mold pressure to which the plants are exposed. It has also been postulated that earlier-flowering plants tend to be erroneously scored as more susceptible in grain mold studies, when they are simply exposed to mold-promoting conditions for a longer period of time than fuller season phenotypes. In those environments in which grain mold pressure was light and occurred late in the season, maturity did not have any affect on the grain mold ratings. In the most severe grain molding environment, early maturity did little to alleviate the damage caused by grain mold. Using the simple linear regression equation of $y = -1.070 + .06483x + \varepsilon$, it can be stated in general terms that for every reduction of approximately two weeks to the date of mid-anthesis, there was a reduction in the grain mold rating of 1.0. This observation contradicts the traditional belief that earliness corresponds to higher levels of grain mold. From a breeding perspective, however, these results are nearly irrelevant. Market factors within both the hybrid seed production and grain sorghum industries dictate a specific range of maturities that are acceptable for both parental lines and the F_1 hybrids they give rise to.

Height is yet another plant characteristic which has been advanced as a potential factor influencing the grain mold reaction of a given plant. The maturing grain on taller plants, especially in a nursery of mixed plant heights, is farther from the soil surface, and, consequently, more protected from the soil-splashed pathogens, high humidity, the

presence of dew, and are more exposed to drying winds than shorter plants. Using the linear regression equation of $y= 8.478 - .0844x + \varepsilon$ (using height as an explanatory variable for grain mold reaction), it can be stated in general terms that for every increase in height of 11.85 inches, there was a predicted reduction of 1.0 in the grain mold rating observed. Market factors would dictate that this cannot be considered as a viable strategy for breeding grain mold resistant sorghum lines.

The grain mold ratings of the original parents used in this experiment provides some perspective on the range of scores to be expected in the progeny, and further serve as a set of standards by which the progeny may be evaluated. Not surprisingly, Sureño greatly exceeded all of the other lines in its capacity to withstand grain mold pressure, with a mean of 2.87 across all locations. At the opposite end of the spectrum was RTx430, with a mean grain mold score of 6.43. The remaining four parents comprised an intermediate class between these two extremes, statistically different from both Sureño and RTx430.

In evaluating the adapted lines as parents, there are various criteria which could be used as determining factors in deciding which were “superior”, in terms of the nature of the progeny that are derived from them. Perhaps the most meaningful among these is simply the mean of the characteristic of interest observed in the progeny. An analysis of variance revealed that the grain mold scores of the progeny of BTx635 were significantly lower ($P<.01$) than those of the other four parents included in this test. The considerable rank shifting apparent in comparing lines per se with the performance of

their progeny indicates that the grain mold reaction of a given line is not always predictive of the expected level of resistance in its progeny.

Several contiguous markers in linkage group E did not follow the expected segregation patterns, and in all cases, the segregation distortion was in favor of the allele originating in adapted parent. Examining the linkage map published by Klein et al. (2001) reveals that QTL for increased plant height associated with linkage group E were discovered. As mentioned above, selection for plants of suitable height for commercial hybrid production was practiced in both the F_2 and F_3 generations. In all likelihood, this selection inadvertently produced an overabundance of the adapted allele among the segregating progeny. The second region that showed some degree of distorted segregation patterns was located on linkage group I. In this case, the overwhelming majority of alleles among the lines originated with Sureño. Although no QTL were detected in the study by Klein et al. (2001) which would point to factors which would have been selected for or against in the earlier generations, it is possible that genes were present within this region which may have contributed to the skewed segregation ratios. Selection for superior agronomic phenotypes was practiced in the F_3 generation, and it is quite possible that Sureño possessed some traits which resulted in the greater numbers of its alleles in this region.

The fact that the QTL were only effective in a cross identical to the original mapping population has some rather important implications for the future development and use of molecular markers for quantitative traits. Whether the results observed here are valid with respect to other quantitative traits remains to be determined, but the

eventual adoption or rejection of this technology by the plant breeding community is contingent upon its ability to effect change across a breadth of germplasm.

REFERENCES

Bandyopadhyay, R., and L. K. Mughogho, 1988. Evaluation of field screening techniques for resistance to sorghum grain molds. Plant Disease 72(6): 500-503.

Bandyopadhyay, R., L. K. Mughogho, and K. E. Prasada, 1988. Sources of resistance to sorghum grain molds. Plant Disease 72(6): 504-508.

Bandyhopadhyay, R., C. R. Little, R. D. Waniska, and D. R. Butler, 2002. Sorghum grain mold: Through the 1990's into the new millenium. pp.173-183. *In*: Leslie, J. F. (ed), Sorghum and Millets Diseases. Iowa State University Press, Ames, IA.

Canez, V. M., and S. B. King, 1981. A comparison of methods for assessing grain mold in sorghum. Phytopathology 71(8): 865.

Castor, L. L., and Frederiksen, R. A., 1980. *Fusarium* and *Curvularia* grain molds in Texas. Pages 92-102 in Sorghum diseases, a world review: proceedings of the international workshop on sorghum diseases, 11-15 Dec 1978, ICRISAT, Hyderabad, India. International Crops Research Institute for the Semi-Arid Tropics: Patancheru, Andhra Pradesh, India.

Chittenden, L. M., K. F. Schertz, Y. –R. Lin, R. A. Wing, and A. H. Paterson, 1994. A detailed RFLP map of *Sorghum bicolor* x *S. propinquum*, suitable for high-density mapping, suggests ancestral duplication of Sorghum chromosomal segments. Theor Appl Genet 87: 925-933.

Dabholkar, A. R., and S. S. Baghel, 1983. Diallel analysis of grain mould resistance in sorghum. Genet. Agr. 37:327-334.

Esele, J. P., R. A. Frederiksen, and F. R. Miller, 1993. The association of genes controlling caryopsis traits with grain mold resistance in sorghum. Phytopathology 83: 490-495.

Flor, H. H., 1956. The complementary genic systems in flax and flax rust. Adv. Genet. 8: 29-54.

Forbes, G. A., R. Bandyopadhyay, and G. Garcia, 1992. A review of sorghum grain mold. Pages 253-264 *in* Sorghum and Millets Diseases: A Second World Review. : ICRISAT: Pantancheru, A.P., India.

Food and Agriculture Organization of the United Nations, Database of agricultural production. FAO statistical databases, 2003. http://apps.fao.org/default.htm

Frederiksen, R. A., D. T. Rosenow, G. L. Teetes, G. C. Peterson, G. N. Odvody, F. R. Miller, and S. D. Collins, 1991. Disease and insect rating schemes for sorghum. Sorghum newsletter 32: 30-36.

Fritz, A. K., A. S. Reddy, S. Pammi, and N. M. Ayres, 1995. Silver staining as a low cost, non-radioactive method of detecting PCR products. p. 184. *In* Agronomy Abstracts, St. Louis, MO.

Glueck, J. A., and L. W. Rooney, 1980. Chemistry and structure of grain in relation to mold resistance. p. 119-140. *In* Sorghum diseases, a world review: proceedings of the international workshop on sorghum diseases, 11-15 Dec 1978, ICRISAT, Hyderabad, India. International Crops Research Institute for the Semi-Arid Tropics:.Patancheru, Andhra Pradesh, India.

Hamada, H., M. G. Petrino, and T. Kakunaga, 1982. Novel repeated element with Z-DNA forming potential is widely found in evolutionarily diverse eukaryotic genomes. Proc. Natl. Acad. Sci. 79:6465-6469.

Harris, H. B., and R. E. Burns, 1973. Relationship between tannin content of sorghum grain and preharvest seed molding. Agronomy Journal 65: 957-959.

Hittalmani S., A. Parco, T. V. Mew, R. S. Zeigler, and N. Huang (2000). Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. Theor Appl Genet 100: 1121-1128.

Huang N., E. R. Angeles, J. Domingo, G. Magpantay, S. Singh, G. Zhang, N. Kumaravadivel, J. Bennett, and G. S. Khush (1997). Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. Theor Appl Genet 95: 313-320.

Ibrahim, O. E., W. E. Nyquist, and J. D. Axtell, 1985. Quantitative inheritance and correlations of agronomic and grain quality traits of sorghum. Crop Sci. 25: 649-654.

Klein, R. R., R. Rodriguez-Herrera, J. A. Schlueter, P. E. Klein, Z. H. Yu, and W. L. Rooney, 2001. Identification of genomic regions that affect grain-mould incidence and other traits of agronomic importance in sorghum. Theor Appl Genet 102:307-319.

Kong, L., J. Dong, and G. E. Hart, 2000. Characteristics, linkage-map positions, and allelic differentiation of *Sorghum bicolor* (L.) Moench DNA simple-sequence repeats (SSRs). Theor Appl Genet 101: 438-448.

Lin, Y.-R., Schertz, K. F., and A. H. Paterson, 1995. Comparative analysis of QTLs affecting plant height and maturity across the Poaceae, in reference to an interspecific sorghum population. Genetics 141: 391-411.

Meckenstock D. H., F. Gomez, D. T. Rosenow, and V. Guiragossian, 1993. Resistration of Sureño sorghum. Crop Sci. 33: 213.

Menkir, A., G. Ejeta, L. Butler, and A. Melakeberhan, 1996. Physical and chemical kernel properties associated with resistance to grain mold in sorghum. Cereal Chem. 73(5): 613-617.

Menz, M. A., R. R. Klein, J. E. Mullet, J. A. Obert, N. C. Unruh, and P. E. Klein, 2002. A high-density genetic map of *Sorghum bicolor* (L.) Moench based on 2926 AFLP, RFLP and SSR markers. Plant Mol. Biol. 48: 483-499. http://sorghumgenome.tamu.edu

Miller, F. R., 1984. Registration of RTx430 sorghum parental line. Crop Sci. 24:1224.

Miller, F. R., 1986. Registration of seven sorghum A- and B-Line inbreds. Crop Sci. 26: 216.

Miller, F. R., R. Clara, and V. Guiragossian, 1992. Registration of A/BTx635 sorghum. Crop Sci. 32:1517.

Miller, F. R., T. F. Dusek, K. L Prihoda, and L. W. Rooney, 1992. Registration of RTx436 sorghum parental line. Crop Sci. 32: 1518.

Miller, F. R., and K. L. Prihoda, 1996. Registration of 16 sorghum germplasm lines: Tx2892 through Tx2906 and Tx2908. Crop Sci. 36: 476.

Mohan, M., S. Nair, A. Bhagwat, T. G. Krishna, M. Yano, C. R. Bhatia, and T. Sasaki, 1997. Genome mapping, molecular markers, and marker-assisted selection in crop plants. Molecular Breeding 3:87-103.

Mukuru, S. Z., 1992. Breeding for grain mold resistance. Pages 273-285 in Sorghum and millets diseases: a second world review. De Milliano, W.A.J., Frederiksen, R.A., and Beggston, G.D., eds. International Crops Research Institute for the Semi-Arid Tropics: Patancheru, A.P, India.

Murty, D. S., K. N. Rao, and L. R. House, 1988. Breeding for grain mold resistance sorghums at ICRISAT. pp154-163. *In* Proc. Internat. Workshop on Sorghum Diseases. ICRISAT, 11-15 Dec., 1978. Hyderabad, India. International Crops Research Institute for the Semi-Arid Tropics: Patancheru, A.P, India.

National Agricultural Statistics Service, 2003. Crop production, 2002 summary. http://usda.mannlib.cornell.edu/reports/nassr/field/pcp-bban/cropan03.pdf

Neter, J., M. H. Kutner, C. J. Nachtsheim, and W. Wasserman, 1996. Applied Linear Statistical Models, 4th ed. Boston: McGraw Hill.

Peng, Y., Schertz, K. F., S. Cartinhour, and G. E. Hart, 1999. Comparative genome mapping of *Sorghum bicolor* (L.) Moench using an RFLP map constructed in a population of recombinant inbred lines. Plant Breeding 118: 225-235.

Pereira, M. G., and M. Lee, 1993. Construction of an RFLP map in sorghum and comparative mapping in maize. Genome 37: 236-243.

Rami, J.-F., P. Dufour, G. Trouche, G. Fliedel, C. Mestres, F. Davrieux, P. Blanchard, and P. Hamon, 1998. Quantitative trait loci for grain quality, productivity, morphological and agronomical traits in sorghum (*Sorghum bicolor* L. Moench). Theor Appl Genet 97: 605-616.

Rao, C. H., and B. S. Rana, 1989. The genetics of characters related to grain deterioration in sorghum. J. Maharashtra Agric. Univ. 14(3): 356-357.

Rodriguez-Herrera, R., W. L. Rooney, D. T. Rosenow, and R. A. Frederiksen, 2000. Inheritance of grain mold resistance in grain sorghum without a pigmented testa. Crop Sci. 40: 1573-1578.

Seitz, L. M., H. E. Mohr, R. Burroughs, and J. A. Glueck, 1983. Preharvest fungal invasion of sorghum grain. Cereal Chem. 60(2): 127-130.

Stuber, C. W., M. Polacco, aand M. L. Senior, 1999. Synergy of empirical breeding, marker-assisted selection, and genomics to increase crop yield potential. Crop Sci. 39:1571-1583.

Thakur, R. P., R. A. Frederiksen, D. S. Murty, B. V. S. Reddy, R. Bandyopadhyay, L. M. Giorda, G. N. Odvody, and L. E. Clafin, 1997. Breeding for disease resistance in sorghum. p. 303-315. *In* Proc Int Conf Genetic Improvement of Sorghum and Millet, 22-27 September 1996, Lubbock, TX.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Van de Lee, M. Hornes, A Frijters, J. Pot, J. Pelman, M. Kuiper and M. Zabeau. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Research. 23: 4407-4414.

Waniska, R. D., J. H. Poe, and R. Bandyopadhyay, 1989. Effects of growth conditions on grain molding and phenols in sorghum caryopsis. Journal of Cereal Science 10: 217-225.

Waniska, R. D., G. A. Forbes, R. Bandyopadhyay, R., R. A. Frederiksen, and L. W. Rooney, 1992. Cereal chemistry and grain mold resistance. p. 265-272. *In* Sorghum and millets diseases: a second world review. De Milliano, W.A.J., Frederiksen, R.A., and Beggston, G.D., eds. International Crops Research Institute for the Semi-Arid Tropics: Patancheru, A.P., India.

Williams, R. J., and K. N. Rao, 1981. A review of sorghum grain moulds. Tropical Pest Management 27: 200-211.

Xu, G. –W., C. W. Magill, K. F. Schertz, and G. E. Hart, 1994. A RFLP linkage map of *Sorghum bicolor* (L.) Moench. Theor Appl Genet 89: 139-145.

Young, N. D., 1999. A cautiously optimistic vision for marker-assisted breeding. Molecular Breeding 5: 505-510.

APPENDIX

Table 43. Tests of between-subjects effects, linkage group D QTL, by location. Abbreviations for the environment are: WES – Weslaco, BEA – Beaumont, CS1 – College Station North, CS2 – College Station South, CA – Corpus Christi Annex, CC – Corpus Christi, VIC – Victoria, and BEE – Beeville.

Dependent Variable: GM

LOC	Source		Sum of Squares	df	Mean Square	F	Sig.
BEA	Intercept	Hypothesis	657.454	1	657.454	400.778	.019
		Error	1.941	1.183	1.640		
	Parent	Hypothesis	9.610	4	2.403	2.808	.031
		Error	66.744	78	.856		
	Replication	Hypothesis	1.784	1	1.784	2.085	.153
		Error	66.744	78	.856		
	Allele	Hypothesis	.374	1	.374	.437	.511
		Error	66.744	78	.856		
	Parent*Allele	Hypothesis	2.110	4	.528	.616	.652
		Error	66.744	78	.856		
BV	Intercept	Hypothesis	3901.567	1	3901.567	524.098	.024
		Error	7.832	1.052	7.444		
	Parent	Hypothesis	28.548	4	7.137	6.431	.000
		Error	89.893	81	1.110		
	Replication	Hypothesis	8.724	1	8.724	7.861	.006
		Error	89.893	81	1.110		
	Allele	Hypothesis	1.288	1	1.288	1.161	.285
		Error	89.893	81	1.110		
	Parent*Allele	Hypothesis	11.453	4	2.863	2.580	.043
		Error	89.893	81	1.110		
CA	Intercept	Hypothesis	1777.975	1	1777.975	3474.063	.000
		Error	1.175	2.295	.512		
	Parent	Hypothesis	17.876	4	4.469	5.034	.001
		Error	67.468	76	.888		
	Replication	Hypothesis	.420	1	.420	.473	.494
		Error	67.468	76	.888		
	Allele	Hypothesis	.518	1	.518	.584	.447
		Error	67.468	76	.888		
	Parent*Allele	Hypothesis	3.425	4	.856	.965	.432
		Error	67.468	76	.888		
CC	Intercept	Hypothesis	992.786	1	992.786	2238.905	.000
		Error	22.451	50.631	.443		
	Parent	Hypothesis	16.997	4	4.249	3.556	.013
		Error	59.743	50	1.195		
	Replication	Hypothesis	5.454E-03	1	5.454E-03	.005	.946
		Error	59.743	50	1.195		
	Allele	Hypothesis	.171	1	.171	.143	.707
		Error	59.743	50	1.195		
	Parent*Allele	Hypothesis	1.284	4	.321	.269	.897
		Error	59.743	50	1.195		

Table 43, Continued

LOC	Source		Sum of Squares	df	Mean Square	F	Sig.
CS1	Intercept	Hypothesis	1266.789	1	1266.789	2946.109	.001
		Error	.810	1.883	.430		
	Parent	Hypothesis	17.842	4	4.460	6.964	.000
		Error	53.161	83	.640		
	Replication	Hypothesis	.383	1	.383	.598	.442
		Error	53.161	83	.640		
	Allele	Hypothesis	1.839	1	1.839	2.872	.094
		Error	53.161	83	.640		
	Parent*Allele	Hypothesis	2.331	4	.583	.910	.462
		Error	53.161	83	.640		
CS2	Intercept	Hypothesis	1264.972	1	1264.972	617.061	.009
		Error	2.818	1.375	2.050		
	Parent	Hypothesis	11.214	4	2.803	2.098	.089
		Error	105.566	79	1.336		
	Replication	Hypothesis	2.258	1	2.258	1.690	.197
		Error	105.566	79	1.336		
	Allele	Hypothesis	.533	1	.533	.399	.529
		Error	105.566	79	1.336		
	Parent*Allele	Hypothesis	5.305	4	1.326	.993	.417
		Error	105.566	79	1.336		
VIC	Intercept	Hypothesis	2184.106	1	2184.106	3010.012	.001
		Error	1.232	1.698	.726		
	Parent	Hypothesis	17.799	4	4.450	4.805	.002
		Error	76.864	83	.926		
	Replication	Hypothesis	.681	1	.681	.735	.394
		Error	76.864	83	.926		
	Allele	Hypothesis	.165	1	.165	.179	.674
		Error	76.864	83	.926		
	Parent*Allele	Hypothesis	2.533	4	.633	.684	.605
		Error	76.864	83	.926		
WES	Intercept	Hypothesis	634.469	1	634.469	2228.163	.001
		Error	.488	1.713	.285		
	Parent	Hypothesis	6.822	4	1.705	4.887	.001
		Error	28.617	82	.349		
	Replication	Hypothesis	.269	1	.269	.772	.382
		Error	28.617	82	.349		
	Allele	Hypothesis	.453	1	.453	1.299	.258
		Error	28.617	82	.349		
	Parent*Allele	Hypothesis	7.425	4	1.856	5.319	.001
		Error	28.617	82	.349		

Table 44. Tests of between-subjects effects, linkage group E QTL, by location. Abbreviations for the environment are: WES – Weslaco, BEA – Beaumont, CS1 – College Station North, CS2 – College Station South, CA – Corpus Christi Annex, CC – Corpus Christi, VIC – Victoria, and BEE – Beeville.

Dependent Variable: GM

LOC	Source		Sum of Squares	df	Mean Square	F	Sig.
BEA	Intercept	Hypothesis	403.049	1	403.049	715.950	.000
		Error	77.231	137.188	.563		
	Parent	Hypothesis	6.538	4	1.635	1.974	.102
		Error	113.463	137	.828		
	Replication	Hypothesis	1.267E-03	1	1.267E-03	.002	.969
		Error	113.463	137	.828		
	Allele	Hypothesis	4.188	1	4.188	5.057	.026
		Error	113.463	137	.828		
	Parent*Allele	Hypothesis	5.198	3	1.733	2.092	.104
		Error	113.463	137	.828		
BV	Intercept	Hypothesis	4157.154	1	4157.154	1221.406	.004
		Error	5.094	1.497	3.404		
	Parent	Hypothesis	21.012	4	5.253	4.305	.003
		Error	173.284	142	1.220		
	Replication	Hypothesis	5.673	1	5.673	4.649	.033
		Error	173.284	142	1.220		
	Allele	Hypothesis	.571	1	.571	.468	.495
		Error	173.284	142	1.220		
	Parent*Allele	Hypothesis	3.331	3	1.110	.910	.438
		Error	173.284	142	1.220		
CA	Intercept	Hypothesis	1942.445	1	1942.445	5608.657	.000
		Error	11.015	31.806	.346		
	Parent	Hypothesis	11.335	4	2.834	4.907	.001
		Error	79.688	138	.577		
	Replication	Hypothesis	.113	1	.113	.196	.659
		Error	79.688	138	.577		
	Allele	Hypothesis	6.775E-05	1	6.775E-05	.000	.991
		Error	79.688	138	.577		
	Parent*Allele	Hypothesis	3.628	3	1.209	2.094	.104
		Error	79.688	138	.577		
CC	Intercept	Hypothesis	1165.899	1	1165.899	1818.993	.000
		Error	31.667	49.406	.641		
	Parent	Hypothesis	17.358	4	4.339	4.046	.005
		Error	89.028	83	1.073		
	Replication	Hypothesis	.142	1	.142	.132	.717
		Error	89.028	83	1.073		
	Allele	Hypothesis	.522	1	.522	.486	.487
		Error	89.028	83	1.073		
	Parent*Allele	Hypothesis	1.766	3	.589	.549	.650
		Error	89.028	83	1.073		

Table 44, continued.

LOC	Source		Sum of Squares	df	Mean Square	F	Sig.
CS1	Intercept	Hypothesis	1291.649	1	1291.649	1694.145	.000
		Error	2.342	3.072	.762		
	Parent	Hypothesis	16.550	4	4.137	6.460	.000
		Error	92.226	144	.640		
	Replication	Hypothesis	.891	1	.891	1.391	.240
		Error	92.226	144	.640		
	Allele	Hypothesis	6.907E-02	1	6.907E-02	.108	.743
		Error	92.226	144	.640		
	Parent*Allele	Hypothesis	4.387	3	1.462	2.283	.082
		Error	92.226	144	.640		
CS2	Intercept	Hypothesis	1231.852	1	1231.852	1301.520	.000
		Error	6.764	7.147	.946		
	Parent	Hypothesis	32.062	4	8.016	6.744	.000
		Error	162.833	137	1.189		
	Replication	Hypothesis	.703	1	.703	.591	.443
		Error	162.833	137	1.189		
	Allele	Hypothesis	12.171	1	12.171	10.240	.002
		Error	162.833	137	1.189		
	Parent*Allele	Hypothesis	3.057	3	1.019	.857	.465
		Error	162.833	137	1.189		
VIC	Intercept	Hypothesis	2153.505	1	2153.505	5306.214	.000
		Error	59.652	146.983	.406		
	Parent	Hypothesis	17.985	4	4.496	5.731	.000
		Error	114.536	146	.784		
	Replication	Hypothesis	6.410E-03	1	6.410E-03	.008	.928
		Error	114.536	146	.784		
	Allele	Hypothesis	1.143	1	1.143	1.457	.229
		Error	114.536	146	.784		
	Parent*Allele	Hypothesis	1.912	3	.637	.812	.489
		Error	114.536	146	.784		
WES	Intercept	Hypothesis	656.417	1	656.417	1449.564	.000
		Error	1.422	3.140	.453		
	Parent	Hypothesis	3.990	4	.998	2.584	.040
		Error	55.991	145	.386		
	Replication	Hypothesis	.523	1	.523	1.354	.246
		Error	55.991	145	.386		
	Allele	Hypothesis	.392	1	.392	1.014	.316
		Error	55.991	145	.386		
	Parent*Allele	Hypothesis	6.356E-03	3	2.119E-03	.005	.999
		Error	55.991	145	.386		

Table 45. Tests of between-subjects effects, linkage group F QTL, by location. Abbreviations for the environment are: WES – Weslaco, BEA – Beaumont, CS1 – College Station North, CS2 – College Station South, CA – Corpus Christi Annex, CC – Corpus Christi, VIC – Victoria, and BEE – Beeville.

Dependent Variable: GM

LOC	Source		Sum of Squares	df	Mean Square	F	Sig.
BEA	Intercept	Hypothesis	462.288	1	462.288	378.472	.007
		Error	1.916	1.569	1.221		
	Parent	Hypothesis	3.011	4	.753	.774	.547
		Error	55.443	57	.973		
	Replication	Hypothesis	1.306	1	1.306	1.343	.251
		Error	55.443	57	.973		
	Allele	Hypothesis	.233	1	.233	.239	.626
		Error	55.443	57	.973		
	Parent*Allele	Hypothesis	.615	4	.154	.158	.959
		Error	55.443	57	.973		
BV	Intercept	Hypothesis	2616.574	1	2616.574	1565.494	.003
		Error	2.534	1.516	1.671		
	Parent	Hypothesis	11.622	4	2.905	2.605	.045
		Error	66.925	60	1.115		
	Replication	Hypothesis	1.890	1	1.890	1.694	.198
		Error	66.925	60	1.115		
	Allele	Hypothesis	3.236	1	3.236	2.901	.094
		Error	66.925	60	1.115		
	Parent*Allele	Hypothesis	8.770	4	2.193	1.966	.111
		Error	66.925	60	1.115		
CA	Intercept	Hypothesis	1246.840	1	1246.840	4865.722	.000
		Error	1.771	6.910	.256		
	Parent	Hypothesis	7.565	4	1.891	3.271	.018
		Error	32.379	56	.578		
	Replication	Hypothesis	.132	1	.132	.228	.635
		Error	32.379	56	.578		
	Allele	Hypothesis	4.801	1	4.801	8.304	.006
		Error	32.379	56	.578		
	Parent*Allele	Hypothesis	1.751	4	.438	.757	.558
		Error	32.379	56	.578		
CC	Intercept	Hypothesis	1104.199	1	1104.199	1348.147	.003
		Error	1.282	1.566	.819		
	Parent	Hypothesis	5.817	4	1.454	1.345	.270
		Error	43.252	40	1.081		
	Replication	Hypothesis	.772	1	.772	.714	.403
		Error	43.252	40	1.081		
	Allele	Hypothesis	2.346	1	2.346	2.169	.149
		Error	43.252	40	1.081		
	Parent*Allele	Hypothesis	7.749	4	1.937	1.792	.150
		Error	43.252	40	1.081		

Table 45, continued.

LOC	Source		Sum of Squares	df	Mean Square	F	Sig.
CS1	Intercept	Hypothesis	915.180	1	915.180	1409.873	.001
		Error	1.252	1.929	.649		
	Parent	Hypothesis	6.555	4	1.639	2.514	.051
		Error	39.116	60	.652		
	Replication	Hypothesis	.648	1	.648	.994	.323
		Error	39.116	60	.652		
	Allele	Hypothesis	.596	1	.596	.914	.343
		Error	39.116	60	.652		
	Parent*Allele	Hypothesis	2.511	4	.628	.963	.435
		Error	39.116	60	.652		
CS2	Intercept	Hypothesis	766.745	1	766.745	616.224	.000
		Error	3.701	2.975	1.244		
	Parent	Hypothesis	7.917	4	1.979	1.566	.195
		Error	74.573	59	1.264		
	Replication	Hypothesis	1.230	1	1.230	.973	.328
		Error	74.573	59	1.264		
	Allele	Hypothesis	1.438	1	1.438	1.138	.290
		Error	74.573	59	1.264		
	Parent*Allele	Hypothesis	3.690	4	.922	.730	.575
		Error	74.573	59	1.264		
VIC	Intercept	Hypothesis	1509.404	1	1509.404	5150.459	.000
		Error	17.819	60.803	.293		
	Parent	Hypothesis	12.215	4	3.054	3.092	.022
		Error	60.239	61	.988		
	Replication	Hypothesis	1.389E-02	1	1.389E-02	.014	.906
		Error	60.239	61	.988		
	Allele	Hypothesis	1.101	1	1.101	1.114	.295
		Error	60.239	61	.988		
	Parent*Allele	Hypothesis	11.230	4	2.808	2.843	.032
		Error	60.239	61	.988		
WES	Intercept	Hypothesis	436.793	1	436.793	4692.465	.000
		Error	5.678	61	9.308E-02		
	Parent	Hypothesis	.675	4	.169	.520	.722
		Error	19.803	61	.325		
	Replication	Hypothesis	.000	1	.000	.000	1.000
		Error	19.803	61	.325		
	Allele	Hypothesis	.799	1	.799	2.461	.122
		Error	19.803	61	.325		
	Parent*Allele	Hypothesis	2.304	4	.576	1.774	.146
		Error	19.803	61	.325		

Table 46. Tests of between-subjects effects, linkage group G QTL, by location. Abbreviations for the environment are: WES – Weslaco, BEA – Beaumont, CS1 – College Station North, CS2 – College Station South, CA – Corpus Christi Annex, CC – Corpus Christi, VIC – Victoria, and BEE – Beeville.

Dependent Variable: GM

LOC	Source		Sum of Squares	df	Mean Square	F	Sig.
BEA	Intercept	Hypothesis	1065.608	1	1065.608	2187.159	.003
		Error	.688	1.412	.487		
	Parent	Hypothesis	5.560	4	1.390	1.551	.192
		Error	103.927	116	.896		
	Replication	Hypothesis	.449	1	.449	.501	.481
		Error	103.927	116	.896		
	Allele	Hypothesis	2.979	1	2.979	3.325	.071
		Error	103.927	116	.896		
	Parent*Allele	Hypothesis	2.459	4	.615	.686	.603
		Error	103.927	116	.896		
BV	Intercept	Hypothesis	6674.537	1	6674.537	1249.526	.015
		Error	5.590	1.047	5.342		
	Parent	Hypothesis	28.493	4	7.123	5.596	.000
		Error	157.835	124	1.273		
	Replication	Hypothesis	5.766	1	5.766	4.530	.035
		Error	157.835	124	1.273		
	Allele	Hypothesis	5.687E-02	1	5.687E-02	.045	.833
		Error	157.835	124	1.273		
	Parent*Allele	Hypothesis	5.224	4	1.306	1.026	.397
		Error	157.835	124	1.273		
CA	Intercept	Hypothesis	2999.758	1	2999.758	26391.508	.000
		Error	.953	8.382	.114		
	Parent	Hypothesis	16.824	4	4.206	6.402	.000
		Error	76.214	116	.657		
	Replication	Hypothesis	4.362E-02	1	4.362E-02	.066	.797
		Error	76.214	116	.657		
	Allele	Hypothesis	5.602E-03	1	5.602E-03	.009	.927
		Error	76.214	116	.657		
	Parent*Allele	Hypothesis	7.719	4	1.930	2.937	.024
		Error	76.214	116	.657		
CC	Intercept	Hypothesis	1984.364	1	1984.364	11151.229	.000
		Error	13.698	76.976	.178		
	Parent	Hypothesis	21.617	4	5.404	4.481	.003
		Error	91.660	76	1.206		
	Replication	Hypothesis	3.116E-03	1	3.116E-03	.003	.960
		Error	91.660	76	1.206		
	Allele	Hypothesis	.667	1	.667	.553	.459
		Error	91.660	76	1.206		
	Parent*Allele	Hypothesis	2.393	4	.598	.496	.739
		Error	91.660	76	1.206		

Table 46, continued.

LOC	Source		Sum of Squares	df	Mean Square	F	Sig.
CS1	Intercept	Hypothesis	2015.950	1	2015.950	2359.567	.007
		Error	1.007	1.179	.854		
	Parent	Hypothesis	15.584	4	3.896	5.629	.000
		Error	85.823	124	.692		
	Replication	Hypothesis	.872	1	.872	1.260	.264
		Error	85.823	124	.692		
	Allele	Hypothesis	1.938E-02	1	1.938E-02	.028	.867
		Error	85.823	124	.692		
	Parent*Allele	Hypothesis	7.159	4	1.790	2.586	.040
		Error	85.823	124	.692		
CS2	Intercept	Hypothesis	1978.409	1	1978.409	2112.674	.001
		Error	1.549	1.654	.936		
	Parent	Hypothesis	11.367	4	2.842	2.110	.084
		Error	157.573	117	1.347		
	Replication	Hypothesis	.861	1	.861	.640	.426
		Error	157.573	117	1.347		
	Allele	Hypothesis	3.234	1	3.234	2.402	.124
		Error	157.573	117	1.347		
	Parent*Allele	Hypothesis	4.810	4	1.203	.893	.471
		Error	157.573	117	1.347		
VIC	Intercept	Hypothesis	3531.295	1	3531.295	5819.073	.003
		Error	.775	1.277	.607		
	Parent	Hypothesis	29.850	4	7.463	10.513	.000
		Error	88.732	125	.710		
	Replication	Hypothesis	.596	1	.596	.839	.361
		Error	88.732	125	.710		
	Allele	Hypothesis	.438	1	.438	.616	.434
		Error	88.732	125	.710		
	Parent*Allele	Hypothesis	10.804	4	2.701	3.805	.006
		Error	88.732	125	.710		
WES	Intercept	Hypothesis	1025.866	1	1025.866	2114.421	.006
		Error	.602	1.240	.485		
	Parent	Hypothesis	5.549	4	1.387	3.548	.009
		Error	48.488	124	.391		
	Replication	Hypothesis	.499	1	.499	1.276	.261
		Error	48.488	124	.391		
	Allele	Hypothesis	6.549E-02	1	6.549E-02	.167	.683
		Error	48.488	124	.391		
	Parent*Allele	Hypothesis	.934	4	.234	.597	.665
		Error	48.488	124	.391		

Table 47. Tests of between-subjects effects, linkage group I QTL, by location. Abbreviations for the environment are: WES – Weslaco, BEA – Beaumont, CS1 – College Station North, CS2 – College Station South, CA – Corpus Christi Annex, CC – Corpus Christi, VIC – Victoria, and BEE – Beeville.

Dependent Variable: GM

LOC	Source		Type III Sum of Squares	df	Mean Square	F	Sig.
BEA	Intercept	Hypothesis	520.891	1	520.891	315.210	.009
		Error	2.559	1.548	1.653		
	Parent	Hypothesis	2.897	2	1.449	1.260	.291
		Error	70.103	61	1.149		
	Replication	Hypothesis	1.851	1	1.851	1.611	.209
		Error	70.103	61	1.149		
	Allele	Hypothesis	2.982	1	2.982	2.595	.112
		Error	70.103	61	1.149		
	Parent*Allele	Hypothesis	7.417	2	3.708	3.227	.047
		Error	70.103	61	1.149		
BV	Intercept	Hypothesis	2739.592	1	2739.592	2344.205	.000
		Error	3.264	2.793	1.169		
	Parent	Hypothesis	4.490	2	2.245	1.672	.196
		Error	87.260	65	1.342		
	Replication	Hypothesis	1.074	1	1.074	.800	.374
		Error	87.260	65	1.342		
	Allele	Hypothesis	2.381	1	2.381	1.773	.188
		Error	87.260	65	1.342		
	Parent*Allele	Hypothesis	4.446	2	2.223	1.656	.199
		Error	87.260	65	1.342		
CA	Intercept	Hypothesis	1321.562	1	1321.562	2287.198	.000
		Error	3.557	6.155	.578		
	Parent	Hypothesis	7.159	2	3.579	3.567	.034
		Error	64.218	64	1.003		
	Replication	Hypothesis	.351	1	.351	.350	.556
		Error	64.218	64	1.003		
	Allele	Hypothesis	3.573E-02	1	3.573E-02	.036	.851
		Error	64.218	64	1.003		
	Parent*Allele	Hypothesis	1.501	2	.751	.748	.477
		Error	64.218	64	1.003		
CC	Intercept	Hypothesis	767.290	1	767.290	1276.654	.000
		Error	8.904	14.815	.601		
	Parent	Hypothesis	13.508	2	6.754	4.968	.012
		Error	50.304	37	1.360		
	Replication	Hypothesis	.207	1	.207	.152	.699
		Error	50.304	37	1.360		
	Allele	Hypothesis	.252	1	.252	.186	.669
		Error	50.304	37	1.360		
	Parent*Allele	Hypothesis	.885	1	.885	.651	.425
		Error	50.304	37	1.360		

Table 47, continued.

LOC	Source		Type III Sum of Squares	df	Mean Square	F	Sig.
CS1	Intercept	Hypothesis	962.908	1	962.908	2982.040	.000
		Error	4.090	12.667	.323		
	Parent	Hypothesis	.620	2	.310	.384	.683
		Error	54.176	67	.809		
	Replication	Hypothesis	.122	1	.122	.150	.699
		Error	54.176	67	.809		
	Allele	Hypothesis	2.868E-02	1	2.868E-02	.035	.851
		Error	54.176	67	.809		
	Parent*Allele	Hypothesis	3.336	2	1.668	2.063	.135
		Error	54.176	67	.809		
CS2	Intercept	Hypothesis	968.410	1	968.410	588.469	.003
		Error	2.979	1.811	1.646		
	Parent	Hypothesis	2.489	2	1.244	.840	.436
		Error	96.304	65	1.482		
	Replication	Hypothesis	1.711	1	1.711	1.155	.286
		Error	96.304	65	1.482		
	Allele	Hypothesis	9.782E-02	1	9.782E-02	.066	.798
		Error	96.304	65	1.482		
	Parent*Allele	Hypothesis	8.516	2	4.258	2.874	.064
		Error	96.304	65	1.482		
VIC	Intercept	Hypothesis	1603.250	1	1603.250	1502.979	.001
		Error	2.022	1.896	1.067		
	Parent	Hypothesis	4.430	2	2.215	2.216	.117
		Error	66.963	67	.999		
	Replication	Hypothesis	1.095	1	1.095	1.095	.299
		Error	66.963	67	.999		
	Allele	Hypothesis	6.673E-02	1	6.673E-02	.067	.797
		Error	66.963	67	.999		
	Parent*Allele	Hypothesis	2.083	2	1.041	1.042	.358
		Error	66.963	67	.999		
WES	Intercept	Hypothesis	470.491	1	470.491	2755.197	.000
		Error	2.891	16.927	.171		
	Parent	Hypothesis	.464	2	.232	.513	.601
		Error	30.310	67	.452		
	Replication	Hypothesis	5.405E-02	1	5.405E-02	.119	.731
		Error	30.310	67	.452		
	Allele	Hypothesis	.125	1	.125	.276	.601
		Error	30.310	67	.452		
	Parent*Allele	Hypothesis	.415	2	.207	.458	.634
		Error	30.310	67	.452		

VITA

Cleve D. Franks, Jr. was born and raised in Houston, Texas. After serving in the Navy during the Gulf War, he attended California Polytechnic State University, San Luis Obispo, where he received a Bachelor of Science Degree in crop science in 1997. Afterwards, he received his Master of Science Degree from Texas A&M University in the field of plant breeding in 2002.

Permanent Address:

4503 81st Street

Lubbock, Texas

79424