

## Abstract

**Jines, Michael Phillip. Identification of Quantitative Trait Loci for Gray Leaf Spot resistance, maturity, and grain yield in a semi-tropical recombinant inbred population of maize. (Under the direction of Major M. Goodman.)**

Identification of QTL can aid in future breeding objectives by allowing breeders either to improve a line through targeted introgressions or assist in forward breeding strategies. Such analyses may be particularly helpful in integrating exotic germplasm into a breeding program. The percentage of tropical maize germplasm grown in U.S. farmers' fields is almost nonexistent. Tropical germplasm in maize (*Zea mays* L.) is a valuable resource to decrease the dependence upon a limited genetic base currently used to produce commercial hybrids, extend selection limits for grain yield, and to provide an insurance function against emerging biotic and abiotic stresses. Results of research presented in this dissertation support these recommendations.

Experiments were conducted to evaluate 143 S<sub>4:5</sub> recombinant inbred lines (RILs) resulting from a cross between NC300, an all-tropical, temperate adapted line, and B104, a stiff stalk line. The 143 RILs were topcrossed to the Lancaster tester FR615xFR697 and randomly subdivided into two sets. The two sets were evaluated for resistance to GLS disease and yielding ability in three and eight North Carolina environments, respectively. Spatial trends were examined in the GLS trials. Significant ( $P \leq 0.01$ ) trend effects were fitted in five of the six set-by-environment combinations, which led to improved analyses within and across environments for both sets.

Ninety-three and eighty-two percent of the RILs in topcrosses (RILT) were significantly ( $P = 0.05$ ) more resistant to GLS when compared to the mean of the commercial checks for set 1 and 2, respectively. Twenty-one RILs from both sets did not

differ significantly ( $P = 0.05$ ) for grain yield when compared to the mean of the commercial checks. RIL 2070 yielded significantly ( $P = 0.05$ ) higher when compared to one commercial check, HC33.TR7322. RIL 1991 was rated the most resistant entry in set 1 and also did not differ from the mean of the commercial checks for grain yield.

The RILs were genotyped at 94 simple sequence repeat loci, and a linkage map was constructed that included nine chromosomes. Composite interval mapping was used to map QTL for GLS resistance, days to fifty-percent pollen shed (DTP), and grain yield. QTL associated with GLS rating were identified at individual environments on chromosomes 1, 4, and 8. One QTL located on chromosome 4 was associated with GLS resistance from the combined analysis across environments and explained 24.5% of the phenotypic variation on an entry-mean basis. A multi-locus model was constructed that involved four marker main effects and a significant epistatic interaction that together accounted for 35.0% of the phenotypic variation. Associations between GLS and maturity have been observed previously. The genetic correlation between GLS rating and DTP in this population was 0.46. A genomic region significantly associated with DTP corresponded to a region associated with GLS resistance on chromosome 8. Further, linked GLS-rating and DTP QTLs occurred together on chromosomes 1, 4, and 8.

QTL associated with grain yield were identified on chromosomes 1, 3, 7, and 9. In the combined analysis across the 2003 environments the NC300 allele had an increasing effect of  $0.15 \text{ t ha}^{-1}$  for the QTL on chromosome 1 and explained 11.0% of the phenotypic variation on an entry-mean basis. The QTL detected on chromosome 3 was from the combined analysis across all environments. The B104 allele had an increasing effect of  $0.20 \text{ t ha}^{-1}$  on chromosome 3 and explained 31.0% of the phenotypic variation.

B104 also had alleles associated with an increasing effect for other grain yield QTLs identified on chromosomes 7 and 9.

These experiments collectively demonstrated the potential that tropical maize germplasm has to improve grain yield and resistance to GLS via conventional or marker-assisted selection strategies.

**IDENTIFICATION OF QUANTITATIVE TRAIT LOCI FOR GRAY LEAF SPOT  
RESISTANCE, MATURITY, AND GRAIN YIELD IN A SEMI-TROPICAL  
RECOMBINANT INBRED POPULATION OF MAIZE.**

By

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## **BIOGRAPHY**

Michael Phillip Jines was born in Bargersville, Indiana, the second child of Michael and Karen Jines. During the summer between his freshman and sophomore year in high school, he was introduced to corn breeding by taking a summer job at Holden's Foundation Seed. Prior to working at Holden's Foundation seed, Michael was interested in civil engineering. Working at Holden's through the remainder of high school prompted Michael to attend Purdue University in West Lafayette, Indiana to major in plant breeding and genetics. At Purdue University, Michael worked with the wheat breeding program under Dr. Herb Ohm. Taking his first plant breeding class, Michael was introduced to Dr. Gebisa Ejeta, a sorghum breeder, and was made aware of the concerns of genetic vulnerability in certain crop species and problems in agriculture of developing countries. The summer before his senior year at Purdue, Michael found his significant other, Brienne Gluvna. Taking the advice of Dr. Ejeta, Michael decided to pursue a Master of Science degree in Crop Science at North Carolina State University, under the direction of Dr. Major M. Goodman. Michael plans to continue his studies in maize breeding at North Carolina State University under the guidance of Dr. Major Goodman to pursue a Ph.D. in Crop Science.

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## **I. Methods Used to Map Quantitative Trait Loci in Inbred Line Cross Populations.**

## Introduction

Quantitative trait variation is the continuous distribution of a character resulting from segregation of many loci whose effects are relatively small when compared to other sources of variation, whereas Mendelian trait variation results in an abrupt, multimodal, distribution as a result of segregation of few loci whose effects are relatively large when compared to other sources of variation (Comstock, 1996). Factors underlying both traits are believed to be inherited by the same forces, as first demonstrated by Nilsson-Ehle (1909) in wheat (*Triticum aestivum*) and East (1916), in *Nicotiana longiflora*. Under the assumption that underlying factors can result in different forms of variation, several people started associating classifiable polymorphisms with that of non-classifiable polymorphisms, based on trait information, and thus began the development of what is currently referred as quantitative trait locus (QTL) mapping.

Payne (1918), using *Drosophila*, was the first to map a QTL, through the use from selected lines, by identification of multiple factors associated with scutellar bristle number on chromosome X. Sax (1923), working with *Phaseolus vulgaris*, identified linkage between a qualitative marker locus, seed pigmentation, and a quantitative trait locus, seed weight. He noticed that dark pigmented seeds of F<sub>2</sub> plants, from a cross between two lines that were in association for heavy and lighter seed weight with dark and light pigmented seeds, respectively, were heavier than non-pigmented seeds. Smith (1937) observed associations between corolla sizes and several independently segregating flower color genes in tobacco. These studies involved measuring a quantitative trait, scoring a qualitative trait, grouping members into classes based on a qualitative trait, and associating classes with trait measurements. Current procedures for a researcher involved

in QTL mapping encompass constructing a linkage map, estimation of QTL position and effects, and, depending on the population, assessing gene action. Therefore, a review of these topics will be presented here.

## **Marker technology**

To begin the search for QTL, one must measure a trait on individuals in a population and genotype these individuals via phenotypic or molecular markers. Falconer and Mackay (1996) described the characteristics of ideal marker loci used in identifying and placing QTLs on a linkage map to be high levels of polymorphism, abundance in throughout the genome, neutrality with respect to both the quantitative trait of interest and to fitness, and co-dominant inheritance. Co-dominance permits complete genotypic classification of individuals, but is not a requirement for mapping.

Phenotypic, or qualitative, markers were not adequate in terms of saturating a genome and were often deleterious with respect to fitness, and further displayed dominant inheritance (Thoday, 1961; Bernardo, 2002). Thus, phenotypic markers were difficult to use to map genes affecting quantitatively inherited traits, and molecular markers were developed to avoid problems associated with phenotypic markers.

Isozymes were the first molecular marker identified (Hunter and Market, 1957).

Isozymes are defined as variants of the same enzyme that possess similar or identical function (Market and Moller, 1959). Although isozymes were useful in genetic studies, only a limited number of loci were polymorphic in any one population (Goodman and Stuber, 1983). Restriction fragment length polymorphisms (RFLPs) replaced isozymes as a marker of choice because they provided more polymorphic loci. Melchinger et al.

(1991) found that RFLP markers were about twice as polymorphic as isozymes in maize. RFLPs reveal variation at the DNA level by producing fragments of DNA of various lengths from restriction digest of genomic DNA, electrophoretically separating fragments by size, and hybridizing the resulting fragments to a radioactive-labeled probe. RFLPs are currently not the marker of choice due to their intense labor and cost requirements (Powell et al., 1996).

Another DNA-based marker involves Simple Sequence Repeats (SSRs), which are repetitive sequences of usually two to three base pairs (Tautz and Renz, 1984). Polymorphisms are revealed from SSRs as a result of amplification by the polymerase chain reaction (PCR) between primers flanking a given SSR (Saiki et al., 1988). SSRs are usually favored over RFLPs because they possess a high rate of polymorphism, are easy to interpret, and do not require as much genomic DNA or radioactive isotope (Taramino and Tingey, 1996; Senior et al., 1996; Smith et al. 1997).

Other PCR based markers include random amplified polymorphic DNA (RAPD; Williams et al., 1990) and amplified fragment length polymorphisms (AFLP; Vos et al., 1995). RAPDs are revealed by PCR amplification of genomic DNA with random primers. AFLPs merge RFLP and PCR technology by subjecting genomic DNA to a restriction digest followed by adapter ligation and PCR amplification of the known adapter sequence. RAPDs and AFLPs are both dominant markers, meaning that they are scored as presence or absence of a band. Further, repeatability of RAPDs and interpretation of AFLPs is of concern.



## Linkage mapping

Marker-trait associations are made possible as a result of linkage. Linkage is the physical attachment of two loci on a chromosome. The magnitude of linkage between two loci is defined by the recombination frequency ( $r$ ), or the frequency of recombinant gametes. Mather (1951) reviewed estimation of recombination frequency using maximum likelihood procedures. The multinomial function is used to obtain the maximum likelihood estimate of  $r$ . Population type, size, and gene action of markers determine how to estimate  $r$ . Population type determines what genotypes are present, and how many recombinant events have occurred. Marker gene action determines the number of observable classes. From this information, the expected probabilities of observable classes, which involve  $r$ , are used to compute the joint probability or likelihood of observing a particular sample of  $n$  genotypes given a value of  $r$ . The likelihood is based on the multinomial formula:

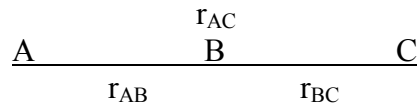
$$L = \frac{n!}{X_1! X_2! \dots X_i!} (m_1)^{X_1} (m_2)^{X_2} \dots (m_i)^{X_i},$$

where  $n$  is the total number of progeny observed,  $i$  is the number of possible classes,  $X_i$  is the number of progeny observed in class  $i$ , and  $m_i$  is the expected proportion of progeny in class  $i$ . To solve for  $r$  once the multinomial formula has been set up, the derivative of the function can be taken with respect to  $r$  and set equal to zero. The value of  $r$  that satisfies the equation can be determined numerically if a direct solution is not available.

Recombination frequencies are not additive because the proportion of recombinant gametes is not linearly associated with the number of crossovers. Therefore, ordering three or more loci on a linkage group can be challenging. Trow (1913) developed a formula that best illustrates this idea. Consider three loci, A, B, and C linked

in the following fashion with corresponding recombination frequencies  $r_{AC}$ ,

$r_{AB}$ ,  $r_{BC}$ :



In Trow's formula (1913),  $r_{AC} = r_{AB} + r_{BC} - 2(1-\delta) r_{AB} r_{BC}$ , where  $\delta$  is the measurement of interference, and ranges from 0 (no interference) to 1 (complete interference). Recombination frequencies are additive only if there is either complete interference, or in the absence of interference, when the term  $2r_{AB}r_{BC}$  approaches zero (i.e. all pair-wise recombination frequencies between loci A, B, and C approach zero).

One goal for obtaining estimates of recombination frequency between markers is to develop a linkage map for each chromosome to assist in the mapping of QTLs. To overcome the non-additive nature of recombination frequencies, several mapping functions have been constructed to help assist in the process of ordering loci when constructing a chromosome map. Mapping functions aim to estimate the total number of crossovers between loci, which are additive, based on observed recombination frequencies and assumptions about interference (Lynch and Walsh, 1998). The unit of mapping functions is cM, which are 100 times the probability of a crossover between two points. The two most frequently used mapping functions are the Haldane and Kosambi mapping functions. The Haldane (1919) mapping function assumes no interference while Kosambi's (1944) mapping function allows for modest interference. Multi-point maximum likelihood linkage mapping is used to order large number of loci on a chromosome, and can be carried out in Mapmaker/exp 3.0b (Lincoln et al., 1993).

## QTL mapping techniques

Once phenotypic and genotypic data have been collected, one must decide what statistical procedure to use to associate phenotype with genotype. Review of theory presented here will assume diploid populations derived from crossing two inbred lines. The simplest model used to test for marker trait associations involves one marker linked to a QTL (Soller, 1976). To determine the expectation for the mean of a particular marker genotype, conditional probability statements are used first to obtain the probability, or frequency, of a QTL genotype given the genotype at a linked marker locus,  $\Pr(Q_j | M_i)$ , where  $M_i$  can be  $M_1M_1$ ,  $M_1M_2$ , or  $M_2M_2$ , and  $Q_j$  can be  $Q_1Q_1$ ,  $Q_1Q_2$ , or  $Q_2Q_2$ . Summing the products of the frequencies and values over all conditional probabilities associated with a given marker genotype yields the expectation for the mean of that particular marker genotype (Lynch and Walsh, 1998; Bernardo 2002). For an  $F_2$  population, the natural estimate of the additive effect is half the difference between homozygous class means, which has expectation:

$$(\mu_{M_1M_1} - \mu_{M_2M_2})/2 = (1-2r)a,$$

where  $\mu_{M_1M_1}$  and  $\mu_{M_2M_2}$  are the two homozygote marker means,  $r$  is the recombination frequency between the marker locus and the QTL, and  $a$  is the additive effect. From this equation, one can deduce whether the marker is linked to a QTL, however, the additive effect,  $a$ , is underestimated by a factor of  $(1-2r)$ . In reality, one does not know the genotype at the QTL, so one simply obtains the mean for a trait for each genotype at a particular marker locus and estimates of additive effects can be obtained through regression or single factor Analysis of Variance (ANOVA), and a t-test is used to declare significance (Soller, 1976; Edwards et al., 1987; Kao et al., 1999). The t-test for the

additive effect is calculated as

$$t = \frac{(\mu_{M_1M_1} - \mu_{M_2M_2})}{\sqrt{\frac{\text{Var}(M_1M_1)}{N_{M_1M_1}} + \frac{\text{Var}(M_2M_2)}{N_{M_2M_2}}}} \quad (\text{Bernardo, 2002}),$$

where  $N_{M_1M_1}$  and  $N_{M_2M_2}$  correspond to the number of individuals with genotypes  $M_1M_1$  and  $M_2M_2$ , respectively.

The single marker model can be further extended through consideration of a mixture model. A mixture model is the “commingling” of multiple distributions, in which each distribution is usually assumed to be normal (Tan and Chang, 1972; Elston, 1974; Boerwinkle, 1986; Hoeschele, 1988). The commingling of multiple distributions results from obtaining the mean for a particular marker genotype. For example, the mean of a marker genotype,  $M_i$ , involves summing over all possible QTL genotype means,  $\mu_{Q_j}$ , at a linked QTL. The phenotype for each  $M_iQ_j$  individual is assumed to be normally distributed with probability density function

$$\phi = [1/(2\pi\sigma^2)]\exp[-(z-\mu_{Q_j})^2/(2\sigma^2)],$$

where  $z$ ,  $\mu_{Q_j}$ , and  $\sigma^2$ , are the phenotypic value, mean, and variance for individual  $M_iQ_j$ , respectively. Therefore, the number of normal distributions associated with each  $M_i$  genotype depends on the total possible  $Q_j$  genotypes at the linked QTL in the population. The variance is assumed to be the same for all individuals (Lynch and Walsh, 1998).

Parameters associated with a mixture model are usually estimated from maximum likelihood procedures (Hasselblad, 1966; Day 1969, Everitt and Hand, 1981; Redner and Walker, 1984; Titterton et al., 1985; McLachlan and Basford, 1988). The likelihood

for an individual given marker genotype  $M_i$ , is

$$l(z|M_i) = \sum_{j=1}^j \phi(z, \mu_{Qj}, \sigma^2) \Pr(Q_j|M_i),$$

where  $z$ ,  $\mu_{Qj}$ , and  $\sigma^2$  are described as before, and the mixing proportions,

$\Pr(Q_j|M_i)$ , are now taken into consideration. The effects of the QTL enter through the  $\mu_{Qj}$ , while the position is obtained from  $\Pr(Q_j|M_i)$ . The total likelihood is the product of the individual likelihoods

$$L(z) = \prod_{n=1}^n l(z_n|M_i),$$

where  $n$  is the number of individuals in the population.

The maximum likelihood estimates of the parameters are the values of the parameters that maximize the likelihood function. The parameter estimates can be obtained through numerical maximization (Gill et al., 1981; Fletcher, 1987) or by iterative procedures, such as the expectation maximization method (Dempster et al., 1977; Harville, 1977; Lynch and Walsh, 1998). For an RIL population, considering a diploid organism, there are four parameters to estimate in the full model, which includes  $\mu_{Q1Q1}$ ,  $\mu_{Q2Q2}$ ,  $\sigma^2$ , and  $r$ , the recombination frequency. The  $L_f(z)$  for the full model is tested against the  $L_r(z)$  of a reduced model. The likelihood for the reduced model is the product of individual likelihoods, however, the only parameters estimated for the likelihood of an individual is the overall mean and variance, thus, disregarding the mixture model.

To test if a QTL is segregating at a particular marker locus, the likelihood ratio test statistic,  $LRT = -2\ln(L_r/L_f)$ , is appropriate (Wald, 1943). The LRT is  $\chi^2$  distributed with degrees of freedom equal to the difference between the number of parameters in the full and reduced models.

The previous single marker locus models do not take into account the information from other linked markers which may be used to obtain a better estimate of the position of a QTL. For example, one could obtain similar results for two markers linked on opposite sides of a highly significant QTL, which could make the placement of a QTL position in an interval between two markers difficult.

Thoday (1961) was the first to suggest the idea of using two markers to bracket a region to test for a QTL. Lander and Botstein (1989) extended the maximum likelihood approach for a single marker linked to a QTL by incorporating Thoday's suggestion, and also by performing a LRT at multiple positions between two linked marker loci. They called this procedure interval mapping (IM). IM can be performed using both maximum likelihood (Lander and Botstein, 1989) and regression procedures (Haley and Knott, 1992; Martínez and Curnow, 1992). The idea of IM is to test a particular position in an interval located between two flanking markers with known map distances. Therefore, a point between the two markers is chosen and conditional probabilities for a QTL genotype given flanking marker genotypes at the position are obtained. Multiple positions between two markers are usually tested at 2.0 cM intervals.

IM performed using maximum likelihood procedures (Lander and Botstein, 1989) follows an approach similar to the single marker case, but the mixture model is extended to account for the conditional probabilities of a QTL genotype at a position, given the two flanking marker genotypes. LRT statistics are obtained for each position tested between the two markers that define an interval, and are usually converted to a logarithm of the odds (LOD) score (Morton 1955). LOD scores corresponding to each position are then

plotted as a function of map position. The position with the highest LOD score is a position that maximizes the likelihood function.

Maximum likelihood estimates of additive and dominant effects are obtained at the position with the largest LOD score in a given interval by performing the appropriate contrast using estimates of the  $\mu_{Qj}$  obtained from the ML procedure. Lander and Botstein (1989) proposed using a one-LOD support interval to obtain approximate confidence intervals for a QTL position. A support interval includes the chromosomal region around a maximum likelihood position that does not differ by a LOD score of one or less when compared to the LOD score of the maximum likelihood position. In contrast, Mangin et al. (1994a,b) demonstrated that a one-LOD support interval is too liberal for QTL with small effects and suggested using a two-LOD support interval.

IM using regression (Haley and Knott, 1992) treats the expected frequencies for the two marker locus genotypes as observations for an explanatory variable, and estimates of additive and dominant effects are obtained via ordinary least squares. The expected frequency of a two locus genotype is obtained by summing over all conditional probabilities for an unknown QTL genotype given the two locus marker genotype. A likelihood ratio test statistic can be constructed as  $LRT = p(SS_{\text{regr}}/SS_{\text{error}})$ , where  $SS_{\text{regr}}$ ,  $SS_{\text{error}}$  are the sums of squares due to regression and error, respectively, and  $p$  is the number of parameters associated with  $SS_{\text{regr}}$ . The position between the two markers that yields the largest LRT and/or  $R^2$ , is the position for a putative QTL. The advantages of Haley and Knott regression includes ease in accommodating other sources of variation entering the model, such as genotype-by-environment interaction, covariates, etc..., and also the reduction in computing resources. A disadvantage using the approach of Haley

and Knott is the estimate of the residual variance is biased upwards, potentially affecting the power of QTL detection (Xu, 1995).

A problem encountered with single marker tests and IM is bias in the estimates of QTL position and effect if there are multiple QTLs linked to an interval being tested (Edwards et al., 1987; Knott and Haley, 1992; Martinez and Curnow, 1992, Zeng 1993, 1994). To overcome the problems associated with multiple QTL, Jansen (1993) and Zeng (1993) independently suggested treating markers outside the interval being tested as covariates. Zeng (1993) called this mapping technique composite interval mapping (CIM).

CIM tests a position similarly as in interval mapping, but other significant positions (co-factors) are accounted for in the model, which can lead to a reduction in the residual variance and result in more power to differentiate between two linked QTL. Co-factors can include positions linked and unlinked to the interval being tested. Others have suggested using regression of marker genotype mean differences on chromosome position to account for the effects of multiple QTL (Kearsey and Hyne 1994; Hyne and Kearsey, 1995; Wu and Li, 1994, 1996). The regression approach uses only markers linked to a QTL in order to address problems related to multiple QTLs. The foundation for the regression procedure considers the expectation for the difference between the mean of the two homozygote marker genotypes, say  $2a(1-2r)$ . A position is chosen along the chromosome and recombination frequencies between all markers and the position are obtained. The recombination frequencies associated with each marker and the position are then placed into the equation  $(1-2r)$ , and these values for each marker become observations for the explanatory variable used in the regression. The observed marker



mean differences are then regressed on the explanatory variable, and the process is repeated several times for different positions along the chromosome. The position that results in the highest  $R^2$ , or smallest SSE, is defined as the position for a putative QTL, and the slope of the regression line should be approximately  $2a$ . The procedure is easily adapted to account for multiple QTLs on a chromosome by using multiple positions.

Single marker analysis and IM, are “one QTL at a time tests”, while CIM is “one QTL and markers” (Kao et al., 1999). Kao and Zeng (1997) and Kao et al. (1999) proposed the idea of using multiple marker intervals simultaneously to identify multiple putative QTL in a model. Kao et al. (1999) called this procedure multiple interval mapping (MIM), and it was shown empirically to be more precise and powerful than compared to IM, and CIM. In addition, MIM performed in QTL cartographer (Wang et al., 2004), allows one to account for epistatic interactions between two positions whose main effects are significant. However, Holland (1997) detected, for several traits in oats, numerous significant epistatic interactions involving at least one locus with no significant main effect. A disadvantage with CIM, marker mean difference regression, and MIM is the inability to discriminate between multiple QTL located in the same interval. Only increasing the sample size will allow one to partition the effects between multiple QTL located in the same interval (Whittaker et al., 1996).

## **Population Comparisons**

Population types derived from inbred line crosses used for QTL mapping include  $F_2$ ,  $F_2$ -derived lines, doubled haploid lines (DHLs), recombinant inbred lines (RILs), and near isogenic lines (NILs). In addition, all of these populations can be topcrossed, and

QTL mapping using marker genotypes of the lines *per se* can be performed on phenotypic data recorded from the topcrosses (Bernardo, 2002; Eathington et al. 1997; Stromberg et al. 1994). Populations can be compared to one another according to numerous criteria, such as efficiency in linkage mapping, estimation of QTL gene action, QTL effect estimation, QTL position resolution, and practical matters. Each population type possesses advantages and disadvantages. Therefore, it is up to the researcher to decide on which population to choose that best suits his or her needs.

Mather (1951), considering two linked Mendelian loci, determined relative efficiencies between  $F_2$  and backcross populations based on the information per individual and found, with co-dominant markers, regardless of linkage phase,  $F_2$  populations are the best for mapping. Backcross populations, using informative markers, are better for linkage mapping than  $F_2$  populations when dominant markers are used to map the population. Liu (1998) extended Mather's approach to incorporate RIL and DHL populations. For values of  $r$  less than 0.15, RIL populations are the best choice for linkage mapping when one only has dominant markers linked in coupling phase. With only dominant markers, DHL and backcross populations have the same relative efficiencies across all values of recombination frequencies and for both linkage phases, provided the recurrent parent used to form the backcross population is recessive at the two marker loci.

If a researcher is interested in determining the gene action of a QTL, then RILs, DHLs, and topcross populations should not be used for QTL mapping, because only additive effects are estimated (Bernardo, 2002). Considering effect estimation for the case of a single marker locus linked to a QTL, DHLs will display a larger additive effect

than RILs (Lynch and Walsh, 1998). The effect estimated for an  $F_2$  population should be larger than RILs. Problems associated with mapping an  $F_2$  population include the inability to replicate individuals.

Advantages of RILs, and DHLs include within-line homogeneity. RILs also allow for greater resolution. Within-line homogeneity can lead to more accurate phenotypic characterization, thus, increasing power to detect an effect at a locus (Lynch and Walsh, 1998). Greater map resolution allows for more accuracy involved in position estimation (Darvasi and Soller, 1995). An example of a high resolution mapping population is the intermated B73 x Mo17 (IBM) population (Lee et al., 2002). The IBM population underwent several rounds of recombination before developing a large number of RILs through selfing.

Typical confidence intervals for QTLs range from 15-20 cM (Lee, M. 1995; Dekkers and Hospital, 2002; Kearsey and Farquhar, 1998). An advantage of NILs allows one to introgress a small donor chromosomal segment into an isogenic background leading to better QTL effect estimation and position localization (Young et al., 1998; Tanksley et al. 1989, Paterson et al. 1991, Dudley 1993). The development of NILs can also allow for fine mapping a QTL by crossing two NILs who share similar introgressed regions, and then derive lines from the cross (Bentolila et al. 1991, Koester et al. 1993, Touzet et al. 1995). Stuber (1999) suggested that obtaining NILs allows one to simultaneously identify QTL and have them introgressed into a homogenized background. Holland (2001) stated that epistasis is rarely observed in RIL populations, where segregation occurs throughout the genome, and the use of NILs containing double

introgressions might be better for examining epistasis, as first demonstrated in barley by Fasoulas and Allard (1962).

## **Conclusions**

QTL mapping performed on a population derived from inbred line crosses has several limitations, regardless of mapping technique and population type. First, limited numbers of recombination events leads to poor resolution for quantitative traits (Flint-Garcia et al., 2003, Dekkers and Hospital, 2002; Kearsey and Farquhar, 1998; Lee, 1995). Secondly, in a diploid organism, only two alleles are sampled in a single population, which impedes the exploitation of the full range of available genetic diversity (Flint-Garcia et al., 2003). Thirdly, the overestimation of effects and percent variation explained that is common in QTL studies using an economical population size can result in overly optimistic projections about the effectiveness of marker assisted selection (MAS) (Beavis, 1998; Melchinger et al., 1998; Utz et al., 2000). Fourthly, results obtained may only be applicable to the defined target environments where phenotypic data were recorded (Stromberg et al., 1994). Fifthly, the uncertainty of marker and target alleles being in high linkage disequilibrium across multiple breeding populations may impede forward breeding schemes (Luby and Shaw, 2001). Finally, QTL effects may change in other backgrounds (Holland, 2001). Addressing any one of these questions means conducting more expensive, and long term experiments. Other techniques such as association mapping (Thornsberry et al., 2001; Flint-Garcia et al., 2003), development of multiple inbred line populations that all share a common parent (Brummer et al., 1997; Kianian et al., 1999; Orf et al., 1999), or construction of lines derived from a diallel cross

(Rebaï and Goffinet 1993, 2000) may allow one to address some of the problems associated with inbred-line cross populations.

Despite these caveats, MAS and marker-assisted backcrossing have been successful in plant breeding in a few cases. These examples include soybean cyst nematode resistance (Cregan et al., 1999), a gene conferring resistance to a wide range of bacterial blight races in rice (Chen et al., 2000), resistance to cereal cyst nematode (Eagles et al., 2001), and resistance to *Fusarium* head blight in wheat (Zhou et al, 2003). Holland (2004) summarized the commonalties between the successful deployments of MAS as having a gene of a large effect, difficulty or expense in measuring a phenotype, close linkage between a marker and the gene, and for forward breeding successes, sources that were introduced from germplasm outside of the programs' breeding pool to maintain linkage disequilibrium between marker and target alleles for future crosses.

A commonality not mentioned was that these examples all involved self pollinating species. In an outcrossing species such as maize, there seem to be limited cases where an economically important trait is controlled by a few genes with a large effect. Marker assisted selection for backcrossing will be beneficial where needed, such as backcrossing transgenes into existing elite lines. Needless to say, keeping in mind the commonalties between the successful deployments of MAS should aide as a road map for choosing future traits to improve via this technology.

## Thesis Background

In maize, little emphasis has been placed on the development of mapping populations whose parentage consisted of a large proportion of tropical germplasm. Marker trait associations identified from such populations might be more applicable for forward breeding schemes for temperate maize breeding. Therefore, a semi-tropical RIL population was derived from a cross involving an adapted, all-tropical, inbred line, NC300, with B104, a stiff stalk line. The two parents were chosen for several reasons. First, outstanding progeny from NC300 have been observed in the NCSU breeding program. Secondly, B104 was chosen because it is a stiff stalk line that offers strong, thick stalks that are necessary for maintaining randomly derived lines as well as good ear quality. The RILs were topcrossed to a sister line tester, FR615xFR697. The Lancaster tester FR615xFR697 is a sister line tester, meaning that both lines are related because they share the same parents. The contrast between two homozygote means at a marker for a RIL population was given by Cowen (1988) as

$$\mu M_1 M_1 - \mu M_2 M_2 = 2a(1-2R),$$

where  $a$  is the additive effect, and  $R$  is the frequency of recombinants observed among the RILs. Haldane and Waddington (1931) derived  $R = 2r/(1+2r)$  to relate  $R$  to the frequency of the recombinant gametes that would be observed between two loci in an  $F_2$  population. The contrast between two homozygote means at a marker locus for RILs topcrossed to an inbred line tester was given by Bernardo (2002), and is the same for an RIL population *per se*, however, the additive effect  $a$  is denoted by  $a_T$ , because phenotypic data is recorded on the topcrosses. Therefore, the amount of heterozygous loci in the sister line tester would depend upon the most recent common ancestor during

the selfing scheme used to generate the two sister lines. Bernardo's equation will be applicable for loci that are homozygous in the sister line tester. When a locus for the sister line tester is heterozygous, then the mean for a particular marker genotype will include a weighted average over the marker/ two QTL genotypes, versus just one in the case if the sister-line tester was homozygous at the locus.

The objectives of this thesis included the identification of lines in topcrosses that are competitive with commercial hybrids for grain yield and agronomic traits, and lines that display resistance to GLS. Secondly, identification of spatial trends for GLS in the experiments involving the RILs in topcrosses was pursued. Further, QTL mapping was performed on the RILs in topcrosses for GLS, maturity, and grain yield. An attempt was made to identify any genomic relationships between GLS and maturity. Finally, QTL associated with GLS resistance and/or maturity from data where plot values were adjusted for spatial trends were compared to QTL identified from a randomized complete block analysis.

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## **II. Dissection of QTL Associated with Gray Leaf Spot Resistance and Maturity in Maize.**

## Abstract

Gray Leaf Spot (GLS) is a foliar disease of maize (*Zea mays* L.) caused by the fungus *Cercospora zeae-maydis*. GLS is recognized as one of the most significant yield-limiting diseases of maize worldwide and has the potential to threaten maize production. Breeding for resistance provides the best preventive measure against the disease. Associations between GLS and maturity have been reported previously. Further, factors such as relative humidity, distance between a plant and an inoculum source, optimum temperature, and light intensity can all affect the rating potential of an experimental plot in a field. 143 S<sub>4.5</sub> recombinant inbred lines (RILs) were developed from single seed descent from a cross between NC300, an all-tropical, temperate adapted line, and B104, a stiff-stalk line. The RILs were topcrossed to the Lancaster tester FR615xFR697 and subdivided randomly into two sets. The two sets were grown at three western North Carolina environments. Each set-by-environment combination was subjected to a trend analysis. Significant trend effects were fitted at five of the six set-by-environment combinations, resulting in reduced standard errors for mean comparisons compared to a traditional randomized complete block design (RCBD) analysis. Entry means from the two analyses were ranked similarly. Combined analyses across environments for both sets where trend effects were fitted at individual environments led to a reduction in both the entry-by-environment interaction variance and the pooled residual variance when compared to the combined analyses across environments that ignored trend effects at individual environments.

Ninety-three and eighty-two percent of the RIL topcrosses (RILTs) were significantly ( $P \leq 0.05$ ) more resistant to GLS when compared to the mean of the



commercial checks for sets 1 and 2, respectively. Composite interval mapping was used to map QTL for GLS rating and days to 50% pollen shed (DTP). QTL associated with GLS rating were identified at individual environments on chromosomes 1, 4, and 8. One QTL located on chromosome 4 was associated with GLS resistance from the combined analysis across environments and explained 24.5% of the phenotypic variation on an entry-mean basis and displayed significant marker-by-environment interaction. A multi-locus model was constructed that involved four marker main effects and a significant epistatic interaction. The multi-locus model explained 35.0% of the phenotypic variation of entry means. The genetic correlation between GLS rating and DTP in this population was 0.46. A genomic region significantly associated with DTP corresponded to a region associated with GLS rating on chromosome 8. Further, linked GLS-rating and DTP QTLs occurred together on chromosomes 1, 4, and 8. Comparisons were made between QTLs associated with GLS rating and DTP from fitting trend effects at individual environments and ignoring trend effects. Minor discrepancies for effect and position were observed between QTLs identified significantly associated with GLS rating and for DTP from the two types of analyses. In one environment, LOD scores for the same QTL associated with GLS rating on chromosome 4 differed by 2.13 between the two different analyses. In the combined analysis across environments for DTP where trend effects were fitted at individual environments, a QTL located on chromosome 5 was found significantly associated with DTP. A corresponding rise in the LOD profile on chromosome 5 occurred from utilizing a RCBD at individual environments, however, positions between presumably the same QTL differed significantly by a two-LOD support interval. Further, the region on chromosome 5 identified in the combined

analysis across environments from utilizing a RCBD at the individual environments did not exceed the genome threshold ( $P = 0.05$ ). These experiments demonstrated that spatial trends were prevalent in a large GLS screening trial and accounting for trend effects can lead to better precision. Also, GLS rating and DTP are somewhat correlated and share similar genomic regions. Results obtained from the identification of QTL when accounting for spatial trends seem to generally coincide with a traditional analysis, however, results occasionally differed.

## Introduction

Gray Leaf Spot (GLS) is a foliar disease of maize (*Zea mays* L.) caused by the fungus *Cercospora zeae-maydis* [Tehon and Daniels, 1925]. GLS is recognized as one of the most significant yield-limiting diseases of maize worldwide (Lipps et al., 1998; Ward et al., 1999). Yield losses associated with GLS result from the reduction of photosynthetic area due to lesions that often coalesce and cause extensive blighting of the leaves. Yield losses are estimated to range between 10 and 25% annually in areas where GLS is endemic, but can be much higher if disease development occurs early enough to impact grain fill and cause severe lodging (Donahue et al., 1991; Donahue, P.J et al., 1986; Jenco, 1995).

*C. zeae-maydis* was first identified in Southern Illinois in 1924 by Tehon and Daniels and was initially confined to the southeastern portion of the U.S. (Tehon and Daniels 1925; Hilty, J. W. et al., 1979). Maize acreage affected by GLS has increased from 7.2 to 14.9 million ha during the 1980s and 1990s (Sparks, 1997). Conservation tillage and monoculture practices have been associated with the proliferation of GLS found throughout maize growing regions in the U.S., resulting from an increase in inoculum present in debris left from the previous growing season (Beckman and Payne, 1983; Payne et al., 1987; Roane et al., 1974; Latterell and Rossi 1977; Ayers et al. 1984; Donahue et al. 1991; Payne and Waldron, J.K. 1983). Currently, GLS is distributed throughout the U.S. as far west as Nebraska, Missouri and Iowa, and east to the Atlantic coast, and has the potential to threaten maize production (Ayers et al., 1984; Hawk et al., 1985; Latterell and Rossi, 1983; Roane et al., 1974; Smith, 1988).

GLS is referred to as a disease of “old age” in maize, because symptoms usually do not develop until plants reach anthesis (Hilty et al., 1979). Rupe et al. (1982) demonstrated the effect of planting date on GLS development by planting the same hybrid at three week intervals. They established that disease development was influenced by physiological age, and demonstrated that a three-week delay in planting date corresponded to a three-week delay in symptom appearance.

Bubeck et al. (1993) estimated Pearson product-moment correlations between days to fifty-percent silk and GLS ratings ranging from 0.12 to 0.27 for different rating times. In addition, Bubeck et al. (1993) observed similar genomic regions governing days to fifty-percent silk and GLS located on chromosomes 2, 3, 4, and 8. Clements et al. (2000) conducted a similar study showing a correlation between GLS rating and the ratio of ear height relative to plant height (ERP). Their results suggested a region located on chromosome one was responsible for controlling both GLS rating and ERP.

GLS is influenced by relative humidity (RH) and leaf wetness (LW). RH and LW determine the growth stage of the fungus. Periods of high RH favor appressoria development and duration of LW may promote spore germination (Beckman and Payne, 1983). Lack of RH and LW encourages sporulation and spore dispersal for the production of secondary inoculum. Rupe et al. (1982) found the average number of trapped spores to be positively correlated with temperature and negatively correlated with RH and LW. Thus, the fungus grows in a vegetative state when RH and LW are favorable and produces spores when conditions are dry. Temperatures in the range of 22 to 30°C promote germination and growth of the fungus (Beckman and Payne, 1983).

*C. zeae-maydis* produces cercosporin (Dunkle and Levy, 2000), a photosensitizing perylenquinone, which disrupts the cellular membrane in the presence of light. Carrera (1996) demonstrated a negative correlation between plant population density and disease severity and hypothesized that increased light resulting from lower population densities may increase the activation of cercosporin. For cercosporin-producing fungi, Daub (2000) demonstrated that light intensities and cercosporin production are required for disease development, and both are linearly associated with disease.

Maturity, RH, LW, temperature, light, amount of initial inoculum, and distance from a plant to an inoculum source are environmental factors that affect the GLS potential of an experimental unit in the field and have been used in modeling procedures to predict grain yield loss (Bhatti and Munkvold, 2002). Such factors are unlikely to be distributed uniformly across experimental fields, and thus spatial trends might be prevalent, which may lead to heterogeneity of within block error variance and poorer precision to detect differences among treatment means. Techniques utilizing alternate analyses have been used extensively for modeling yield potential according to soil heterogeneity (Brownie, 1993; Bhatti, 1991; Bowman, 1990; Casler, 1999; Kempton, 1994). Models that account for spatial variation in two dimensions have been demonstrated to be more efficient when compared to an analysis utilizing a randomized complete block design (RCBD) (Gusmão, 1986; Lin et al., 1993). However, selecting an appropriate model can be challenging because different models tend to rank entries differently (Brownie et al., 1993).

Control of GLS can be achieved through several approaches, including residue management, crop rotation, use of fungicides in the benzimidazole and triazole chemical

groups, and resistant hybrids (Ward et al., 1997). The integration of these management practices can be deployed to effectively control GLS. However, resistant hybrids offer the best protection against GLS for several reasons. For example, residue management can only control the proliferation of *C. zea-maydis* in the area one is trying to manage, but adjacent properties may or may not be involved in such management practices. Secondly, conservation tillage has major benefits for the environment and producer. Also, the implementation of fungicides may not be profitable or environmentally desirable.

Roane and Genter (1976) evaluated 193 commercial hybrids and 541 inbreds and experimental lines for GLS resistance. Their results suggested only several inbreds and 4% of the hybrids examined demonstrated resistance to GLS. Ayers et al. (1984) characterized most elite germplasm in the U.S. as being susceptible to *C. zea-maydis*. Resistance to GLS usually comes from the Lancaster side of the pedigree for commercial hybrids (Ulrich et al., 1990; Carson, 2002). Presently, some commercial hybrids with increased tolerance to GLS have been developed. However, all genotypes eventually show some symptoms given adequate levels of inoculum and appropriate environmental conditions. Sources found to be resistant include material of largely tropical origin (Bubeck et al., 1993; Kraja and Dudley, 2000; Holland and Goodman, 1995). Some commercial hybrids currently have adequate resistance, but few early hybrids have much resistance.

The inheritance of resistance to GLS has been studied through biometrical procedures (Roane and Genter, 1976; Manh, 1977; Ayers et al., 1984; Huff et al., 1988; Thompson et al., 1987; and Ulrich et al., 1990). All studies indicated that resistance to

GLS was primarily additive in nature, with a few exceptions of non-additive gene action (Elwinger, et al., 1990; Gevers et al., 1994).

Since the advent of molecular markers, numerous QTL associated with GLS resistance have been identified. Bubeck et al. (1993) detected QTL on all chromosomes of maize from three different populations. One region on chromosome 2 was associated with GLS resistance in all three of the populations, and significant QTL by environment interactions were observed. Saghai Maroof et al. (1996) identified QTL on chromosomes 1, 4, and 8 that had large effects on GLS resistance, explaining 35.0-56.0%, 14.3%, and 7.7-11.0% of the variance, respectively. Clements et al. (2000) found five QTL located on chromosomes 1, 2, 5, and 7 significantly associated with GLS resistance. Further, these five regions were consistent across environments. Lehmensiek et al. (2001) found five QTL associated with GLS resistance on chromosomes 1, 3, and 5. Gordon et al. (2004) identified two QTL associated with GLS resistance on chromosomes 2 and 4.

The maize-breeding program at North Carolina State University has focused on the development of inbred lines derived primarily from tropical sources and devotes considerable efforts in screening such materials for resistance to GLS. Unfortunately, we find that resistant genotypes tend to be later maturing. Therefore, we developed a semi-tropical recombinant inbred line (RIL) population, consisting of 143 lines, and topcrossed the RILs to a Lancaster tester to address the following questions.

First, what spatial trends, if any, are present in GLS screening trials involving the RIL topcrosses (RILT), and how do these trends affect the analysis involving the ranking of entries at a location and across environments for a large GLS screening trial? To date, no analysis accounting for spatial trends has been examined for foliar diseases of maize.

How do the RILT compare in terms of GLS resistance to a wide range of commercial hybrids grown in North Carolina?

Secondly, we wanted to identify QTL for GLS resistance and maturity in the RILT population. Do any of the identified QTL for GLS correspond to similar or dissimilar genomic regions in previous studies that identified QTL for GLS resistance? Do QTL identified for GLS severity based on entry means across all environments remain significant when conducting the analysis on entry means at each location (i.e. is QTL-by-environment interaction prevalent?). Also, which parent possesses favorable alleles for QTL associated with GLS resistance?

Thirdly, we wanted to assess the magnitude of the correlation between DTP and GLS resistance in the RIL population. In addition, what genomic regions are accountable for an association between these two traits? Are genomic regions that confer both resistance and later maturing phenotypes a result of linkage or pleiotropy? Finally, how does the trend analysis compare to an analysis using a RCBD to generate entry means for the detection of significant genomic regions associated with GLS resistance, and also for DTP?



## **Materials and Methods**

### **Development of the Population**

A semi-tropical recombinant inbred population was developed from a cross between NC300 and B104. It was initiated by Dr. Terry Molnar and was provided by Dr. Major M. Goodman at North Carolina State University. NC300, an all-tropical, temperate adapted line, is a second cycle line derived from a cross between two experimental lines derived from hybrids PioneerX105A and PioneerX306B x H5, respectively. Pioneer X105A and Pioneer X306B are two double cross tropical hybrids developed in Jamaica during the 1970's. Pioneer X105A consists of Cuban Flint, ETO, and Tuxpeño germplasm, and Pioneer X306B possesses Chandelle, Tusón, Coastal Tropical Flint, and Cuban Flint germplasm. H5, a double-cross, tropical hybrid, contains Cuban Flint and Tuxpeño germplasm and was developed by the CNTA program in El Salvador. For more information about inbred lines developed from these tropical hybrids, refer to Holley and Goodman (1988). B104 is a temperate line developed at Iowa State University and is derived from BS13(S)C5-13-1 (Hallauer et al., 1997). The BS13 is a strain of BSSS that has undergone 12 cycles of recurrent selection, primarily for grain yield.

The initial cross was generated in the winter nursery in 1998 in Homestead, Florida.  $F_1$  seed was planted the following spring in the greenhouse and was self-pollinated to produce  $S_0$  seed.  $S_0$  plants were grown in the Raleigh 1999 summer nursery. After self pollinating 143  $S_0$  plants, seed from each  $S_0$  plant was used to plant one row in Homestead, Florida in 1999. The  $S_{0:1}$  rows were self-pollinated, and seed from one of the ears from each  $S_{0:1}$  row was sampled to plant an entire row in the next

generation. Each successive generation was subjected to this type of treatment until 143 randomly derived  $S_{4.5}$  RILs were established with an inbreeding coefficient equivalent to  $F=0.96875$  (Cockerham, 1983).

### **Experimental design**

The 143 RILs were topcrossed to the Lancaster tester FR615xFR697, a C103 sister line cross (MBS, 2001). The 143 RILs were then assigned at random to one of two sets, and the experimental design was a replication within sets design. Each set was replicated two times at a location. Treatments, or RILs and commercial checks, are referred to as entries. Set one consisted of eighty-one entries including sixty-seven RILs and twelve commercial hybrid checks. Set two consisted of ninety entries including seventy-six RILs and twelve commercial hybrid checks. Both sets shared the same commercial checks, and each set included NC300 and B104 topcrosses to FR615xFR697. A susceptible commercial check included in both sets was Pioneer 3394.

Each set was grown adjacent to one another in each of three western North Carolina locations in 2003 for GLS evaluation. Locations included were Andrews, Laurel Springs, and Salisbury, NC, and are referred to as the GLS environments. All three environments are conducive for GLS development. However, plots were only planted in corn debris from the previous year at Andrews and Salisbury. The source of inoculum for Laurel Springs was infested oat grains provided by Syngenta, and was applied at the V6 stage of growth.

Experimental units consisted of a single row, 20-plant plot, 4.86 m in length at Laurel Springs and Salisbury. Eighteen plants per entry were assigned to a plot 3.05 m in length at Andrews. A 1.0 m alley was located at the end of a plot at Laurel Springs, and

Salisbury. At Andrews, a 0.8 m alley was located at the end of a plot. Inter-row spacing at Laurel Springs, Salisbury, and Andrews was 0.91 m, 0.76 m, and 0.76 m, respectively. Therefore, plant density at Laurel Springs, Salisbury, and Andrews was 45,222, 54,147, and 73,770 plants ha<sup>-1</sup>, respectively. Plots were subjected to standard North Carolina cultural practices.

Visual GLS ratings were taken on a plot basis (i.e. the visual average of all plants in a plot) using a one to nine scale, with one designated as susceptible and nine as resistant. Resistance was defined as the ability for a given entry to retard colonization of the host by the fungi, but colonization of plants eventually occurs. Allocations of ratings to plots were according to Bubeck et al. (1993) and were based upon the amount of overall lesions, spread of lesions from the lower to the upper leaves of the plants in a plot, and if lesions coalesced and became necrotic.

All three environments were planted on May 15, 2003. Ratings were initiated at a time when the majority of plots at an environment had reached anthesis. Once ratings commenced at an environment, subsequent ratings were taken at approximately 10 day intervals. Five ratings were taken at Andrews on August 8, August 18, September 1, September 11, and September 20 in 2003. Four ratings were taken at Laurel Springs on August 22, September 1, September 12, and September 22 in 2003. Due to the confounding effects of southern rust (caused by *Puccinia polysora* Underw.) late in the season, only three ratings were taken at Salisbury on August 16, August 26, and September 6.

Days to fifty percent pollen shed (DTP) were recorded at Andrews and Laurel Springs, and were defined as the interval between the number of days when a plot was

planted and fifty percent pollen shed. In addition, DTP measurements were also obtained in two non-GLS environments. The same sets were grown at Clayton, NC in 2002, and 2003. Plot dimensions differed for the two Clayton locations when compared to the GLS environments. Specifically, a plot at either the Clayton 2002 or 2003 location consisted of a two-row plot, 4.86 m in length including a 1.2 m alley at the end of the plot. Row spacing was 0.97 m; 44 plants were allocated to each plot. Therefore, plant density for the two Clayton locations was 46,660 plants ha<sup>-1</sup>.

Entries present in the Clayton 2002 location differed from the GLS environments and Clayton 2003 location by the number of checks in both of the sets. Further, the Clayton 2002 location had fewer experimental entries. Specifically, the Clayton 2002 location had one and two fewer RILT for set one and set two, respectively. Thus, set one for Clayton 2002 consisted of eighty-one entries, seventy-five RILT and six commercial checks, and set two consisted of seventy-two entries, sixty-five RILT, and seven commercial checks. The experimental design for both sets at the Clayton 2002 and 2003 locations was a RBCD with two and three replications, respectively, and for subsequent analyses involving DTP as the response variable, a year and location combination was considered a single environment.

### **Statistical analysis for phenotypic data**

Plot values for GLS ratings at a given GLS environment were estimated by taking the arithmetic average of all ratings included at an environment, following the findings of Saghai Maroof (1993). Ratings involved in the estimation of plot values at an environment incorporated the first rating at a given environment that displayed distinct differences in GLS reactions between known susceptible and resistant entries, and all

subsequent ratings taken at an environment. Therefore, five ratings from Andrews, three ratings from Laurel Springs, and three ratings from Salisbury were retained to obtain plot values for GLS ratings at these environments, respectively.

In the analyses for GLS ratings and DTP, entries were treated as fixed effects since we were interested in this specific set of RILT. The commercial checks were retained for adjusting the RILT for set effects. Entry means were first obtained from an Analysis of Variance (ANOVA) utilizing a randomized complete block design (RCBD). The model for the RCBD included the block and the residual variance, block-by-entry interaction, as random effects.

Separately, a trend analysis, or polynomial regression was used (Brownie et al., 1993 ; Federer and Schottfeldt, 1954 ; Kirk et al., 1980 ; Tamura and Naderman, 1988). The trend analysis involved adjusting plot values in a set according to spatial trends in an attempt to reduce the effects of heterogeneity within blocks. Adjustment of plot values was based upon the position of a plot in the grid defined by the rows and columns of the field layout (Appendix II). Row and column effects were modeled using first through fourth order orthogonal polynomials obtained by a macro written in PROC IML in SAS v 8.0 (SAS institute, Cary, N.C.). The  $n^{\text{th}}$  degree polynomial coefficients associated with row and column for a plot were termed trend effects, and treated as fixed effects. Initially, a model including entry and the seven trend effects for a given environment and set was termed the “full model”, and involved the following terms:

$$Y_{ij} = \mu + \tau_i + \beta_{rk} X_{ijk} + \beta_{cl} P_{ijl} + \epsilon_{ij} \quad (\text{eq. 1})$$

Where  $\mu$  is the overall set mean,  $Y_{ij}$  is the response for the  $j^{\text{th}}$  plot assigned to entry  $i$ ,  $X_{ijk}$  is the  $k^{\text{th}}$  degree polynomial coefficient corresponding to row, assigned for the  $j^{\text{th}}$  plot

assigned to entry  $i$ ,  $P_{ijl}$  is the  $l^{\text{th}}$  degree polynomial coefficient corresponding to column, assigned for the  $j^{\text{th}}$  plot assigned to entry  $i$ ,  $\beta_{rk}$  is the regression coefficient for row corresponding to the  $k^{\text{th}}$  degree polynomial coefficient, and  $\beta_{cl}$  is the regression coefficient for column corresponding to the  $l^{\text{th}}$  degree polynomial coefficient, and  $\epsilon_{ij}$  are random errors associated with plot  $j$  assigned to entry  $i$  with  $E(\epsilon_{ij})=0$ . The levels of  $k$  and  $l$  were four and three, respectively. Blocks were not included in the trend analysis because including a term for blocks would result in a model for response potential with arbitrary discontinuities along block boundaries, whereas trend analysis assumes the response potential to vary in a smooth manner (Brownie et al., 1993).

Choosing an appropriate model including only significant trend effects and the entry main effect involved the utilization of PROC MIXED in SAS v 8.0 (SAS institute, Cary, N.C.). The following algorithm was used to choose an appropriate model. First, a model including only entries was fitted and residuals were plotted against rows and columns to visually display any apparent trends. Next, all terms were fitted for the “full model”, equation 1. Interaction effects between all factors were not included in any of the model building steps due to the geometric increase of terms in the model.

To avoid over-fitting a model, only significant trend effects ( $P \leq 0.01$ ) from the full model were included in a “reduced” model, along with the entry main effect (Brownie et al., 1993). If trend effects in the reduced model remained significant, then model building was considered finished, and the best model was chosen from the trend analysis. However, if an effect that was significant in the full model became non-significant in a reduced model, the process of elimination was repeated until a model was established in which all remaining trend effects were significant ( $P \leq 0.01$ ).

The trend analysis and ANOVA utilizing the RCBD were compared to each other on the basis of precision. Precision was defined by the standard errors of differences among entry means (Brownie et al., 1993). Therefore, standard errors were calculated for differences among all pair-wise combinations of entry means for both analyses. The analysis that provided the smallest average standard error (ASE) value for differences among entry means was considered the best model, and entry means from the best model were used for QTL detection.

Spearman rank correlation coefficients (SRCC) for GLS rating were calculated between entry means obtained from the analysis incorporating trend effects and the analysis utilizing the RCBD for a given set and environment, and also for each set across environments by using the SPEARMAN statement in PROC CORR in SAS v 8.0 (SAS institute, Cary, N.C.). Least significant differences were also calculated in SAS v 8.0 using the TINV function for each environment and set combination for the trend and RCBD analyses.

An across environments analysis was conducted for each set based on entry means from within each environment. A pooled error term for GLS rating for each set was constructed by taking a weighted average of the residuals across all environments for each analysis method and adjusted for use in the combined analysis (Milliken and Johnson, 1992). Entry-by-environment interactions for each set obtained from the combined analysis across environments were tested against the corresponding pooled error terms.

Fisher's protected LSD (Steel et al., 1997) was used for entry mean separation for GLS rating and DTP; entries in different sets were analyzed separately. An ANOVA was

performed using a model for GLS rating including environment and entry as random and fixed effects, respectively. Entry-by-environment interaction was used as the error variance because the analysis for a set was based upon entry means from individual environments obtained from either the trend or RCBD analysis on the basis of precision. The entry-by-environment interaction term also served as the error term used for the calculation of the LSD.

Entry means for GLS rating and DTP from the two different sets were obtained and adjusted for set effects within and across environments (Schutz and Cockerham, 1962). Adjusting entries for set effects for the two levels involved a similar process following Schutz and Cockerham (1962). Entry means from separate sets at the same environment obtained from the ANOVA utilizing either the RCBD or trend analysis were adjusted for sets by first obtaining deviations for entry means over replications from the corresponding set mean over replications at an environment. Then, the mean of the two sets was added to these deviations.

Obtaining entry means across environments adjusted for set effects for GLS rating and DTP was performed using PROC MIXED in SAS V8.0 utilizing the LSMEAN statement (SAS institute, Cary, N.C.). An ANOVA, involving the same model, was used for both response variables with the only exception of excluding non-common entries in the Clayton 2002 environment and the remaining three environments for DTP. Particularly, the ANOVA for the combined analysis across environments for GLS rating and DTP involved a model that included set, environment, and set-by-environment interaction terms as random effects. Entry nested within set was considered a fixed effect. The entry-by-environment nested within set interaction term served as the



residual variance in the model, because the analysis was based upon entry means obtained from each set and environment combination based on precision according to either the RCBD or trend analysis. Entry means from the combined analysis across environments adjusted for set effects for GLS rating and DTP were obtained by subtracting the corresponding set mean across environments, and then adding the mean of both sets across environments to these deviations.

### **Genetic Parameters**

Plot-basis and entry mean-basis heritability estimates were obtained for GLS rating and DTP. The target environments for GLS ratings included the three GLS environments. Target environments for DTP included two GLS environments where maturity was recorded, and also the Clayton 2002 and 2003 environments. Commercial checks, NC300 and B104 topcrosses were excluded from the data set used to estimate heritability. Further, non-common RILT among the environments used to estimate heritability for DTP were dropped from the data set. The model used to estimate heritability for GLS rating and DTP followed Hallauer and Miranda (1988), and included environment, set, environment-by-set interaction, block nested within environment-by-set interaction, entry nested within set, and entry-by-environment nested within set interaction as random effects. Entry nested within set effect was treated as random here because we were interested in the estimation of the among entry variance component. The block-by-entry nested within environment-by-set interaction served as the residual variance for the model.

Heritability estimates were obtained using a program written by Holland et al. (2003) in PROC MIXED in SAS V 8.0 (SAS institute, Cary, N.C.). The delta method

(Lynch and Walsch, 1998) was utilized to derive approximate standard errors for both heritability estimates. Heritability for DTP involved using the harmonic mean for the total number of experimental units assigned to a given RILT, because there were two replications at the GLS and Clayton 2002 environments and three replications at the Clayton 2003 environment (Holland et al., 2003).

Genetic and phenotypic correlations for GLS and DTP at the Andrews and Laurel Springs environments were estimated. A multivariate analysis of variance (MANOVA) was performed to obtain mean cross products for entry nested within set, and the entry-by-environment nested within set interaction. The block-by-entry nested within environment-by-set interaction served as the residual variance. The MANOVA was performed using the REPEATED statement in PROC MIXED in SAS v 8.0 (SAS institute, Cary, N.C.). Similarly, the delta method (Lynch and Walsch, 1998) was utilized to derive approximate standard errors for the genetic and phenotypic correlation estimates. Pearson product moment correlation coefficients (PPMC) based on plot values were estimated between each GLS rating taken at the Andrews and Laurel Springs environments, and DTP using PROC CORR in SAS v 8.0 (SAS institute, Cary, N.C.). Pearson product moment correlations were also calculated between GLS rating and DTP based on entry means adjusted for sets across all environments where each response variable was recorded.

### **Genotyping the RILs and Linkage Map Construction**

Four leaf tissue samples were harvested from four different plants for each of the 143 S<sub>4;5</sub> RILs, *per se*, and for NC300 and B104, when the plants were 3 to 4-wk-old.

DNA was extracted according to Riede and Anderson (1996) with slight modifications (see appendix VII).

DNA suspended in Tris-CL EDTA (TE) buffer for the four samples corresponding to the same RIL was bulked. The RIL and two parents were genotyped using 94 simple sequence repeat (SSR) markers in an attempt to achieve coverage at 20 cM intervals. SSR reaction procedures, conditions, and gel scoring were based on Senior et al. (1996, 1998). For further detail, please see appendix VIII. Four percent agarose gels were set up to include one well each for NC300 and B104 reaction products as well as the 142 RILs. The RIL that had the lowest genomic DNA concentration was dropped in order to fit the two parents and the population on 1.5 PCR plates in order to maximize usage of PCR machines. Digital images of gels were taken using an Eagle Eye II Still Video System (Stratagene, La Jolla, CA). Gels were scored at a given SSR marker locus by assigning a 1 or 2 corresponding to the presence of NC300 and B104 genotypes, respectively. Heterozygous lines were assigned a 3.

RILs found to be heterozygous at a given marker locus were eliminated in the construction of a linkage map and in subsequent analyses involving QTL detection for that particular locus. Pearson's Chi square goodness of fit statistic (Snedecor and Cochran, 1980) was used to test if markers deviated significantly ( $P \leq 0.01$ ) from a 1:1 segregation ratio by using a macro written in Proc Freq in SAS v8.0. Marker loci that displayed segregation distortion were not included originally in the construction of a linkage map. Mapmaker/exp 3.0 (Lincoln et al., 1993) was employed to construct a linkage map using a logarithm of the odds (LOD) threshold of 3, a 50 cM distance, and the Haldane mapping function. Marker placement was compared to the IBM2 2004

neighbors map [www.maizedb.org](http://www.maizedb.org) to identify any deviations in locus ordering. Markers that displayed significant segregation distortion prior to map construction were included in the final map if their placement coincided to the IBM2 2004 neighbors map.

### **QTL Detection**

Phenotypic data sets (PDS) were each merged with the molecular marker data set. PDS included entry means for RILT adjusted for set effects for GLS rating and DTP obtained from analyses executed for separate environments, and across environments. Further, PDS incorporated entry means for RILT adjusted for set effects for GLS rating and DTP obtained from analyses executed for the separate environments, and across environments utilizing entry means obtained from the analysis incorporating the RCBD for a given set and environment.

Composite interval mapping (CIM) was used to estimate the additive effects of genomic regions for all PDS, and was performed using QTL-Cartographer (Wang, 2001-2004). A genome-wise significance threshold ( $P = 0.05$ ) for each phenotypic data set was obtained through permutation testing following Churchill and DeGeorge (1994). Permutation testing involved 1,000 iterations for all phenotypic marker datasets. Both backward and forward selection procedures were used to perform the permutation testing and CIM, using a threshold of 0.01 for factors to enter and remain in the model. The genome was scanned every 2.0 cM. Confidence intervals were constructed for all significant genomic regions by using a 2-LOD support interval following Mangin et al. (1994a,b). Markers identified significantly associated with GLS rating were subjected to a multi-factor ANOVA in PROC GLM in SAS V 8.0 (SAS institute, Cary, N.C.) to test for marker-by-environment interaction. The model included environment, set,

environment-by-set, rep nested within environment-by-set, and marker-by-environment nested within set as random effects while marker nested within set was considered a fixed effect.

A multi-locus model was constructed using multi-factor ANOVA in PROC GLM in SAS V 8.0 (SAS institute, Cary, N.C.) on all marker main effects that were identified from CIM as significantly associated with GLS rating from any of the trend phenotypic data sets. All pair-wise marker combinations were tested for the presence of epistasis for GLS rating through the usage of the software EPISTACY, written in SAS v 8.0 (Holland, 1998). Significant ( $P \leq 0.001$ ) marker-by-marker interaction terms were involved in the multi-marker locus modeling. Markers were retained in the model if the main effect was significant ( $P \leq 0.01$ ), or if a marker was involved in a significant ( $P \leq 0.001$ ) interaction.

## Results

### Comparison of trend analysis versus RCBD analysis for GLS rating

Set 1 means for GLS rating at the Andrews, Salisbury, and Laurel Springs environments were 6.49, 6.94, and 7.02, respectively. Mean GLS rating at the Andrews, Salisbury, and Laurel Springs environments for set 2 was 6.26, 6.90, and 7.14, respectively. ASE values obtained from the analysis for a given set and environment, and also for the combined analysis across environments, where trend effects were fitted at individual environments, were lower when compared to ASE values obtained from the corresponding RCBD analysis (Table 1). Consequently, trend analyses were more precise when compared to the corresponding RCBD analysis for five of the six set-by-environment combinations and also for the combined analyses for both sets. The only set and environment combination where trend effects were not significant was set 1 at Laurel Springs.

The coefficients of variation for a given set and environment from the two models followed the same patterns as ASE values.  $R^2$  and F-values for testing entry main effects were higher from trend analyses than with the corresponding RCBD analysis. Accordingly, LSDs ( $P=0.05$ ) obtained from the trend analysis for a given set and environment, and across environments, were lower when compared to LSDs obtained from the corresponding RCBD analysis.

Plots of residuals versus columns for the six set-by-environment combinations are presented in Figures 1 through 3. No row trend effects were fitted for GLS rating in any of the models for the different environment-by-set combinations. Further, plots involving residuals versus rows tended to be invariable among rows (data not shown). Linear

column effects were fitted at the Andrews and Salisbury environments in addition to quadratic, cubic, and quartic effects. A quadratic column effect was fitted at the Laurel Springs environment in set 2.

Table 2 displays Spearman rank correlation coefficients for GLS rating based on entry means obtained from the two analyses for a given set and environment, and the combined analyses across environments. SRCC were high across all set and environment combinations. The Laurel Springs set 2 environment had the lowest SRCC, whereas, the SRCC from the combined analysis across environments for the two separate sets had the highest SRCC.

Entry-by-environment interactions for the two sets were significant ( $P \leq 0.002$ ). Entry-by-environment interaction mean squares for both sets tended to be smaller in the combined ANOVA when trend effects were fitted at individual environments than compared to the combined ANOVA where plot values at individual environments were not adjusted for trend effects (Table 3). Pooled error terms in both sets were smaller in the combined ANOVA when trend effects were fitted at individual environments than in the combined ANOVA that ignored trend effects. As a result, entry-by-environment interactions were more significant in the combined ANOVA across environments than compared to the combined ANOVA where trend effects were ignored at individual environments. Furthermore, the entry main effects were more significant in the combined ANOVA across environments when trend effects were fitted at individual environments than the combined ANOVA that ignored trend effects.

### **Entry Mean Separation for GLS rating**

GLS was prevalent in 2003 (Table 4). The entry mean for GLS rating across environments for P3394 was 4.06 and 4.25 for sets 1 and 2, respectively. The overall experiment mean for GLS rating for sets 1 and 2 was 6.82 and 6.77, respectively. The mean GLS rating across environments for the RILT in sets 1 and 2 was 6.99 and 6.89, respectively. Entry mean GLS ratings for the RILT ranged from 5.98 to 7.83 in set 1 and from 5.39 to 7.81 in set 2. The mean of the commercial checks across environments for sets 1 and 2 was 5.91 and 5.97, respectively. Entry means for the commercial checks across environments ranged from 4.06 to 6.83 in set 1 and from 4.25 to 6.75 in set 2. The mean of the two parents in topcrosses across environments for GLS rating was 6.55 and 6.73 for sets 1 and 2, respectively.

P3394 was the significantly lowest entry across environments for GLS rating in both sets ( $P = 0.05$ ). The highest GLS entry mean across environments was RILT 1991-T10 in set 1 and 2096-T10 in set 2. The GLS entry mean across environments for B104-T10 in sets 1 and 2 was 5.35 and 5.75, respectively. The GLS entry mean across environments for NC300-T10 in sets 1 and 2 was 7.75 and 7.70, respectively. No transgressive segregates were observed in either set with respect to either parents. Sixty-two out of sixty-seven RILT were significantly higher when compared to the GLS mean of the commercial checks in set 1 ( $P = 0.05$ ). Sixty-two out of seventy-six RILT in set 2 were significantly higher when compared to the GLS mean of the commercial checks. Forty of the RILT had a significantly higher GLS rating when compared to the mean of the two parents in set 1 ( $P = 0.05$ ). Twenty-five of the RILT had a significantly higher GLS rating when compared to the mean of the two parents in set 2. Subsequently,



twenty-six and thirty-two of the RILT in sets 1 and 2, respectively, did not differ significantly when compared to NC300 in topcrosses ( $P = 0.05$ ).

### **Entry Mean Separation for DTP**

The overall experiment means for DTP for sets 1 and 2 were 75.51 and 75.36, respectively (Table 5). The DTP means across environments for the RILT in sets 1 and 2 were 75.58 and 75.36, respectively. DTP entry means for the RILT ranged from 73.62 to 77.92 in set 1 and from 73.76 to 77.72 in set 2. The mean of the commercial checks for DTP across environments for sets 1 and 2 was 74.79 and 74.89, respectively. Entry means for the commercial checks across environments for GLS rating ranged from 72.73 to 76.43 in set 1 and from 72.80 to 76.38 in set 2.

HC33.TR7322 flowered the earliest among all entries in set 1. Entries 1989-T10, 2016-T10, and G8288 present in set 1 did not differ significantly for DTP when compared to HC33.TR7322 ( $P = 0.05$ ). For set 2, HC33.TR7322 flowered the earliest. However, six RILT and G8288 did not differ significantly for DTP when compared to HC33.TR7322 ( $P = 0.05$ ). Two out of sixty-five of the RILT in set 1 flowered earlier than the mean of the commercial checks ( $P = 0.05$ ). Relative DTP in 2003 environments for B104 and NC300 topcrosses were 76 and 78 days, respectively (data not shown). RILT 2026-T10 in set 2 flowered significantly earlier than the mean of the commercial checks ( $P = 0.05$ ). Twenty-six RILT in set 1 flowered significantly ( $P = 0.05$ ) later than the mean of the commercial checks, and twenty-two out of seventy-five RILT in set 2 flowered significantly later than the mean of the commercial checks ( $P = 0.05$ ).

## **Genetic Parameters and Pearson Product-Moment Correlations**

Plot and entry mean heritability estimates for GLS rating and DTP, phenotypic and genetic correlations between the two traits, and their standard errors are displayed in Table 6. Both plot and entry mean heritability estimates were slightly higher for GLS rating than for DTP. PPMC for plot values between each GLS rating taken at an environment and DTP were all significant ( $P \leq 0.001$ ), and tended to increase with later rating dates in both sets at Andrews, but remained relatively static at Laurel Springs (data not shown). PPMC at Andrews ranged from 0.14 to 0.46 across rating times. PPMC at Laurel Springs ranged from 0.21 to 0.37 across rating times. The PPMC for the average of GLS ratings included at Andrews and DTP was 0.42. PPMC for the average of GLS ratings included at Laurel Springs and DTP was 0.25. The PPMC for entry means across all environments adjusted for sets between GLS rating and DTP was 0.38 ( $P \leq 0.0001$ ).

## **Linkage Map**

A linkage map was constructed that included 94 markers on nine chromosomes and is displayed in appendix I. Only three markers were obtained on chromosome six, and there was not enough information to construct a linkage map for that chromosome. The length of the map of the nine chromosomes was 1821.1 cM, with an average distance between markers of 19.37 cM. Therefore, an estimated 90% of the genome was covered if one assumes a quality map in maize is around 2000 cM (Senior et al., 1996). Nine percent of the genotypic data were missing, including heterozygotes. Nine markers that deviated significantly ( $P \leq 0.05$ ) from a 1:1 segregation ratio were included in the linkage map; umc1335 (chromosome 1), bnlgl017 (chromosome 2), umc1587 (chromosome 3),

phi113 (chromosome 5), three markers located between positions 0.0 and 34.9 cM on chromosome 7, and umc2099 and phi22 (chromosome 9). Further, marker locus ordering was in agreement when compared to the IBM2 2004 neighbors map.

### **GLS QTL**

Table 7 displays information on position,  $R^2$  values, effects, and LOD scores for QTL identified significantly ( $P = 0.05$ , genome wise error rate) associated with GLS resistance from the various PDS. QTL reported for the various PDS for GLS rating, and DTP are from CIM performed on entry means where trend effects were fitted at the individual environment(s), because fitting trend effects at the individual environment(s) improved precision.

The allele from NC300 had an increasing effect for all putative QTL found significantly associated with GLS rating. One QTL associated with GLS resistance on chromosome 4 from the combined analysis across environments is referred to as GLSQTL1. GLSQTL1 was located in the interval between marker bnlg1265 and bnlg1621 and explained 24.5% of the phenotypic variation of entry means with an effect of 0.264. The GLSQTL1 was found significantly associated with GLS rating at the Andrews and Salisbury environments where trend effects were fitted. The GLSQTL1 is designated as ANDGLSQTL1 for the Andrew environment and SALGLSQTL1 for the Salisbury environment. Further, alleles from NC300 had an increasing effect at all environments, i.e. there was no change in rank between the effects of the alleles from the two parents. The ANDGLSQTL1 and SALGLSQTL1 explained 7.0 and 22.0 % of the phenotypic variation, respectively.

Another QTL that was adjacent to ANDGLSQTL1 on chromosome 4 was found significantly associated with GLS rating in the Andrews environment, and is designated as ANDGLS2QTL. ANDGLSQTL2 was located in the interval between marker umc2082 and umc1117 and explained 10.8% of the phenotypic variation with an effect of 0.250. In addition to the SALGLSQTL1 identified significantly associated with GLS rating at the Salisbury environment, another QTL located on chromosome 8 was identified for GLS rating and is designated as SALGLSQTL2. The SALGLSQTL2 was located in the interval between marker umc1360 and umc1034, and explained 13.4% of the phenotypic variation with an effect of 0.199.

One QTL on chromosome 1 was associated with GLS resistance in the Laurel Springs environment and is designated as LASGLSQTL1. The LASGLSQTL1 was located on chromosome 1 in the interval between marker umc1071 and bnlgl47, and explained 23.9% of the phenotypic variation with an effect of 0.273. LOD score plots for the QTL identified in only one environment had non-significant LOD score peaks at the other environments and for the combined analysis across environments (data not shown). Further, no change in rank occurred between the effects of the alleles from the two parents across environments. Only markers flanking the GLSQTL1 displayed a significant ( $P < 0.01$ ) marker-by-environment interaction.

### **Maturity QTL**

Table 8 displays information on position,  $R^2$  values, effects, and LOD scores for the various DTP PDS. Four QTL were found significantly associated with DTP from the combined analysis across environments where trend effects were fitted at each of the individual environments. The four QTL were located on chromosomes 1, 4, 5, and 8.  $R^2$

values ranged from 0.09 to 0.18. DTP and GLS rating QTL occurred together on chromosomes 4 from the combined analysis for these two traits (Figure 4). Further, two non-significant LOD score peaks for GLS rating from the combined analysis occurred on chromosome 8 (Figure 5). One of the non-significant peaks corresponded to the SALGLSQTL2, whereas the other non-significant peak corresponded to a QTL for DTP.

The QTL identified significantly associated with DTP on chromosome 4 is referred to as DTPQTL1. Markers that flanked DTPQTL1 were umc1043 and umc1051. The NC300 allele had an increasing effect at this position. The DTPQTL1 position from the combined analysis was also found significantly associated with DTP at all other individual environments, except at Laurel Springs.

The QTL identified significantly associated with DTP on chromosome 8 from the combined analysis across environments is referred to as DTPQTL2. Markers that flanked DTPQTL2 were umc1562 and bnlg2181. The allele that had an increasing effect at the DTPQTL2 position was that of NC300. Further, the DTPQTL2 position was found significantly associated with DTP at the Laurel Springs and Clayton 2003 environments.

An additional occurrence between one region corresponding to LASGLSQTL1 from the combined analysis across environments, and another region significantly associated with DTP, occurred on chromosome 1 (Figure 6). The QTL identified significantly associated with DTP is referred to as DTPQTL3. The DTPQTL3 was flanked by marker bnlg2228 and umc2047. The allele with an increasing effect was from NC300.

### **Multi-locus modeling for GLS rating**

A significant ( $P \leq 0.0001$ ) epistatic interaction occurred between markers bnlgl1175 and umc2182. Bnlgl1175 is located on the short arm of chromosome 2, and umc2182 is located 5.2 cM from marker umc1562, the marker flanking the region for DTPQTL2 on chromosome 8. Considering DTP, the NC300 genotype at marker bnlgl1175 had a non-significant ( $P = 0.2332$ ) increasing effect of 0.09 days, whereas, at marker umc2182 the NC300 genotype had a non-significant ( $P = 0.0271$ ) increasing effect of 0.20 days (data not shown). The model for GLS rating involving the two marker main effects and the interaction explained 20% of the phenotypic variation. The additive main effect for marker bnlgl1175 and umc2182 was 0.11 ( $P = 0.0066$ ) and 0.09 ( $P = 0.0250$ ), respectively (Table 9b). Mean GLS rating for the four classes constituting the interaction had overlapping standard errors, except for the mean of individuals who had B104 genotypes at both marker loci, which had the lowest mean GLS rating (Table 9a).

Correspondingly, for both loci, the NC300 genotype had a non-significant negative effect when judged in the background of the NC300 genotype at the other locus. The NC300 genotype at a locus had a significant ( $P = 0.0001$ ) increasing effect when judged in the background of the B104 genotype at the other locus.

The multi-locus model constructed utilizing multi-factor ANOVA involved markers bnlgl1621, bnlgl147, bnlgl1175, umc2182, and bnlgl1175-by-umc2182 interaction; and accounted for 35.0% of the phenotypic variation (Table 10). The main effects for markers bnlgl1175 and umc2182 were not significant ( $P > 0.01$ ). The two marker main effects that flanked the ANDGLSQTL2 were not significant ( $P > 0.01$ ) when bnlgl1621 was in the model. The marker main effect for umc1360, one of the markers that flanked

SALGLSQTL2, was significant ( $P = 0.0010$ ) when bnlgl621 was in the model.

However, when including bnlgl175, umc2182, and their corresponding interaction, the main effect for umc1360 was not significant ( $P > 0.01$ ). A model including only bnlgl621 was significant ( $P \leq 0.0001$ ), and explained 15.0% of the phenotypic variation.

### **Comparisons between the TREND analysis and RCBD for QTL detection**

Positions,  $R^2$  values, and effects were similar for a QTL obtained from fitting trend effects at individual environment(s), and not fitting trend effects at individual environment(s) for GLS rating from performing CIM on entry means obtained at an individual environment, or from the combined analyses. Positions changed between the two combined analyses for a QTL associated with DTP on chromosome 5. The combined analysis across environments where trend effects were fitted at individual environments placed the DTP QTL 58 cM closer to the short arm of chromosome 5 than the DTP QTL identified from not fitting trend effects at individual environments. The two positions differed significantly, considering a 2 LOD support confidence interval (data not shown), and were located in two different marker intervals.

Flanking markers were the same for GLS rating QTL identified from fitting trend effects at the environment(s) and not fitting trend effects at corresponding environment(s). Flanking markers for the QTL identified on chromosome 4 for DTP differed between fitting trend effects at the Clayton 2003 environment and not fitting trend effects at the Clayton 2003 environment. Markers umc1043 and umc1051 from the trend analysis, and markers umc1051 and umc1940 from the RCBD analysis flanked the QTL located on chromosome 4 for DTP at the Clayton 2003 environment. The two

positions obtained did not differ significantly considering a two LOD score support interval (data not shown).

Discrepancies were apparent for LOD scores from CIM for both GLS rating and DTP between fitting and not fitting trend effects at the individual environments, and also for the two combined analyses. The LOD score for the SALGLSQTL1 from the trend and RCBD analyses differed by 2.13 (Figure 7). The LOD score for the SALGLSQTL2 from the trend and RCBD analyses differed by 0.56 (Figure 8). The LOD score for the LASGLSQTL1 from the trend and RCBD analyses differed by 0.31 (Figure 9).

For DTP, one QTL was significant only in one of the two types of combined analyses across environments. The chromosomal region on chromosome 5 was significantly associated with DTP from the combined analysis across environments where trend effects were fitted at individual environments. But, it was not significantly associated with DTP in the combined analysis across environments where trend effects were not fitted at individual environments (Figure 10). The LOD score between the two types of analyses differed by 0.59.



## Discussion

Trends appeared in a large GLS screening trial involving the RILT population, as has been found in other traits and in other crop species (Bhatti, 1991; Brownie, 1993; Bowman, 1985; Kempton, 1994; and Casler, 1999). Adjusting for trend effects at five of the six set-by-environment combinations improved precision in terms of comparing entry means for a set at a particular environment as indicated by lower LSDs obtained from fitting trend effects. “Escapes” are common when screening genotypes for resistance to a particular disease (Louie and Anderson, 1993). If escapes are physically clustered in the field, accounting for spatial effects should improve phenotypic characterization of individuals.

No row trend effects were fitted in any of the spatial modeling. Fitting only column trend effects could be due to the layout of the plots for a particular set. Specifically, the three GLS environments for each set had at most four rows, or ranges, and the column dimension was roughly 15 to 20 times the size as the number of rows. Therefore, row effects may become of importance if the GLS environment field dimensions were made longer, and not as wide.

Even though the number of rows for a particular set at an environment was around 4, both sets were grown adjacent to one another according to the length of the field. Further, residuals were unrelated to row positions for both sets, indicating uniform disease pressure across one axis of the field according to the length of the field. Typical GLS rating fields are used repeatedly each year in order to build up adequate levels of inoculum. If similar trend effects were observed over years for different environments

and different material, such knowledge might be beneficial in terms of field layout and blocking design.

High SRCC between entry means obtained from the RCBD and trend analyses at a particular environment indicate that entries were ranked in a similar fashion between both analyses. SRCC were similar between the two sets within a given environment, because similar trend effects were fitted for the 2 sets at the same environment. The SRCC between entry means obtained from the two combined analyses for GLS rating were higher than compared to SRCC obtained at any of the individual environments.

Several factors were improved for both sets in the combined analyses across environments performed on adjusted GLS rating entry means according to trend effects at individual environments when compared to the conventional corresponding combined analysis. Such factors included smaller pooled error estimates, smaller entry-by-environment interaction variances, and larger F-values for testing the entry main effect. Consequently, entry-by-environment interaction terms were more significant when adjusting for trend effects at individual environments. Entry-by-environment interaction has been previously documented in prior experiments as a significant source of variation for GLS rating (Carson et al., 2002; Bubeck et al., 1993; Clements et al.; and Saghaii et al., 1996).

The RILT, as a whole, were some of the highest-rated materials the NCSU maize breeding program has screened. For example, some of the RILT ranked highest among a large number of commercial hybrids we screen annually for the North Carolina Corn Grower's association. NC300, *per se*, is usually rated as a 7 for GLS resistance, and

B104 a 6. Furthermore, the sister line tester, FR615xFR697, is rated as a 6. Thus, it is not surprising that the RILT, as a whole, were noticeably more resistant than other materials.

All favorable alleles corresponding to QTL identified significantly associated with GLS rating were contributed from NC300. Consequently, no transgressive segregates were observed with respect to either parent in the topcrosses. One putative QTL was found on chromosome 4 in the combined analysis across environments. The QTL located on chromosome 4 explained 24.5% of the phenotypic variation, and had an effect of 0.264, which explained around 13% of the range of the RILT.

In addition, the QTL located on chromosome 4 was significantly associated with GLS rating in the Andrews and Salisbury environment. The same chromosomal region was not significantly associated with GLS resistance at the Laurel Springs environment. Nonetheless, a peak in the LOD profile at Laurel Springs occurred at this same region. Subsequently, there was no occurrence of a change in rank between the two alleles contributed by the two parents at Laurel Springs for the chromosome 4 QTL.

The SALGLSQTL2, located on chromosome 8, was significantly associated with GLS rating only at the Salisbury environment. However, in all other environments there was no apparent change in rank between the alleles contributed between the two parents. In addition, there was no change in rank between the two alleles across the other PDS for the LASGLSQTL1 located on chromosome 1.

A multi-locus model was constructed from the combined analysis across environments that contained markers identified from CIM that were significantly associated with GLS rating corresponding to GLSQTL1, and LASGLSQTL1. In addition, marker bnlgl1175 (chromosome 2) and umc2182 (chromosome 8) were included

in a multi-locus model due to the significant interaction between the two loci. The epistatic interaction observed between marker bnlgl175 and umc2182 is further supported by the higher mean GLS rating observed among the RILT than compared to the mean of the parents for both sets, which would result from epistasis.

GLS and DTP were somewhat correlated. The genetic correlation between GLS rating and DTP reported for the RILT population was 0.4557. Further, PPMC for GLS rating and DTP at Andrews and Laurel Springs environments were 0.40 and 0.29, which were higher than reported in other studies (Bubeck et al., 1993; Rupe et al., 1982; and Manh et al., 1977; Gordon et al. 2003).

DTP and GLS rating were only recorded at the Andrews and Laurel Springs environment; however, several other environments for each trait were used to further dissect the relationship between maturity and GLS resistance. A genetic correlation can arise from several reasons such as gametic phase disequilibrium, linkage disequilibrium, and pleiotropy (Falconer and Mackay, 1996). The DTPQTL1 found on chromosome 4 as well as GLS1QTL and ANDGLSQTL1 from the combined and individual environment analyses, respectively, seem likely to cause a correlation due to linkage.

Two peaks occurred for GLS rating on chromosome 8 from the combined analysis, and both peaks were slightly less than the threshold of 2.89. From the combined analysis, a concomitant increase in the LOD profile for GLS rating occurred corresponding to DTPQTL2 on chromosome 8. Marker umc2182, involved in the interaction term from the multi-locus model was linked 5.4 cM from marker umc1562, one of the markers that flanked DTPQTL2. Essentially, the mean GLS rating of individuals who possessed B104 genotypes at both markers involved in the interaction

were scored significantly lower than compared to the other three classes comprising the interaction. Neither marker bnlgl175 nor umc2182 were significantly associated with DTP, however. The NC300 genotype had a non-significant increasing effect at both loci, and individuals possessing a B104 genotype at both loci flowered earlier than compared to the other three classes. For chromosome 1 from the combined analysis, LOD profiles between GLS rating and DTP resembled one another at the LASGLSQTLL1 position. Further, the DTPQTLL3 occurs on the same chromosome.

Twenty-four lines possessed NC300 and B104 genotypes at marker bnlgl1621 and umc1051, respectively. Bnlgl1621 corresponds to GLSQTLL1, whereas, umc1051 corresponds to DTPQTLL1. Both markers are located on chromosome 4. Of the twenty-four, three RILs performed favorably in topcrosses for grain yield when compared to the mean of the commercial checks. The three lines include RILs 1990, 1991, and 1999. In addition, RILs 1990 and 1991 possess B104 genotypes at DTPQTLL2 and DTPQTLL3 (appendix VI). Five double recombinants were observed at GLSQTLL1. Two RILs, 1971 and 2080, had a favorable recombination for the NC300 allele on chromosome 4. Mean GLS rating across environments for RILT 1971-T10 and 2080-T10 was 7.19 and 7.37, respectively, suggesting the resistance allele was retained in the double crossover event. RILs 1971 and 2080 could be used to transfer the GLSQTLL1 chromosomal region into B104 via backcrossing.

Comparisons can be made to other studies on the basis of QTL map positions, as localized on the IBM2 2004 neighbors map. The GLS1QTL concurs with that of the Bubeck et al. (1993) study. In the Bubeck et al. (1993) study, seven markers were significantly associated with GLS rating on chromosome 4. More striking is the

concurrence between DTPQTL1 and similar regions found on chromosome 4 that were significantly associated with GLS ratings in other studies. As previously mentioned, Bubeck et al. (1993) identified similar regions associated with GLS rating and DTP that agree with DTPQTL1 when compared to the IBM2 2004 neighbors map. Further, a region identified on chromosome 4 for GLS rating by Saghai Maroof et al. (1996) and Gordon et al. (2004) corresponds to the region for DTP1QTL.

Another striking agreement between the results of this study and that of others is the agreement of a significant region associated with DTP on chromosome 5 from the combined analysis in this study that corresponds to the results of significant regions associated with GLS rating of other studies. For example, Bubeck et al. (1993), Clements et al. (2000), and Lehmensiek et al. (2001) identified GLS QTL on chromosome 5 that map to the region on chromosome 5 from the combined analysis that was significantly associated with DTP. Similarly, the QTL significantly associated with GLS rating on chromosome 8 in Clements et al. (2000) and Saghai Maroof et al. (1996) corresponds to the DTP2QTL region.

Similar chromosomal positions were not significantly associated with both GLS rating and DTP on chromosomes 4, 5, and 8 in the RILT population. A lack of overlapping regions significantly associated with both DTP and GLS rating could result from recording GLS rating on RILs topcrossed to a semi-resistant tester, causing RILT to all have the same genotypic value for GLS rating at the various DTP loci. Also, inaccurate phenotypic characterization of some plots could have resulted from segregation of the tester alleles.

Consequently, comparisons made to other studies are speculative, because similarities between significant regions associated with DTP in this study to significant regions associated with GLS rating observed in other studies could result from other reasons, such as different alleles in the mapping populations, inbreds versus hybrids, etc. Nonetheless, when mapping QTL for GLS rating, one should also include an extensive set of environments where some measurement of maturity is recorded. In the previous studies involved in mapping QTL for GLS rating, maturity was recorded in only one environment in two different studies. Gordon et al. (2003) measured maturity, but did not map QTL associated with maturity. Further work is needed to molecularly characterize these relationships.

Results from CIM between fitting trend effects at individual environment(s), versus ignoring trend effects at individual environment(s) for both GLS rating and DTP yielded similar results. For example, positions,  $R^2$  values, and additive effects were of similar magnitude between the two types of analyses, except for the DTP QTL on chromosome 5 from the combined analyses. However, LOD scores differed between some of the analyses for the two traits. LOD scores indicated that the SALGLSQTL1 obtained from CIM on entry means from the trend analysis was 100 times more likely than the QTL estimated from the RCBD analysis, which is large enough to be of concern. Further, the DTP QTL on chromosome 5 was only declared significant in the combined analysis across environments where trend effects were fitted at individual environments.

At an individual environment, when the trend analysis produces a higher LOD score than the RCBD analysis, the trend analysis is adjusting plot values in a fashion that reduces the within marker variability relative to the among marker variability and should

increase the probability of QTL detection. Contrarily, when the RCBD analysis produces a higher LOD score than the trend analysis, the trend analysis is adjusting plot values in a fashion to reduce the among marker variability relative to the within marker variability.

Otherwise, results from CIM between the two types of analyses are similar as a result of several levels of “buffering”. First, a better estimate of an entry mean is obtained as the number of experimental units an entry is assigned increases. Secondly, for an RIL population, 50% of the RILs should be of one genotype and the other 50% of the RIL population should be of the other genotype. If RILs are randomly assigned to plots, then adjusting plots according to trend effects should not effect the estimation of the additive effect provided genotypes at a particular locus are non-systematically allocated to plots across any apparent spatial trends. However, adjusting for trend effects can alter the pooled within marker variance, or the likelihood of no QTL in the model, by reducing the heterogeneity of the within block error variance which leads to better estimation of entry means. Inconsistencies of estimated QTL effects across environments could result from either genotype-by-environment interaction, or within environmental variation. Methods that lead to better reduction of the latter should be pursued where appropriate.

In summary, one QTL was identified significantly associated with GLS resistance across environments from CIM. Further, the QTL is located on chromosome 4 and should offer a new source of resistance as it comes from NC300, a line unrelated to U.S. commercial maize. In addition to the QTL identified on chromosome 4, a significant epistatic interaction was detected that was comparable in terms of the amount of explained phenotypic variation as the chromosome 4 QTL. The development of a



mapping population resulting from a cross between an RIL that possesses alleles with an increasing effect for both GLS rating and DTP on chromosome 8 and B104 may prove beneficial for fine mapping to further dissect maturity and GLS resistant factors (Bentolila et al. 1991, Koester et al. 1993, Touzet et al. 1995). Further, accounting for trend effects at individual environments can alter the results of CIM, however, apparent differences between the RCBD and trend analyses would have not affected any marker assisted selection strategies, except for the DTP QTL found on chromosome 5. Several papers, suggested increasing sample size of mapping populations to better obtain estimates of effects and percent variation explained (Beavis, 1998; Gilles, 2000; Melchinger, 1997). A larger sample size mandates better field plot techniques. Subsequently, examining the effects of spatial trends on QTL expression may lead to a better understanding of inconsistencies of QTL across environments. Finally, both plot and entry mean heritability estimates for GLS rating and DTP were high, indicating that selection for markers may prove beneficial only if one is trying to execute selection in environments where GLS is not prevalent.

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Table 1. Trend analysis summary for each set and environment combination and the combined analysis across environments (ACR) for GLS rating.

Analysis	Set	Env	MR	FV	CV	R <sup>2</sup>	LSD	ASE	SETM	DF	Effects
RCBD	1	AND	0.22	6.47	7.28	0.87	0.94	0.47	6.49	80	
Trend	1	AND	0.17	8.23	6.40	0.90	0.83	0.42	6.49	80	C1,C3
RCBD	2	AND	0.27	6.06	8.32	0.86	1.04	0.52	6.26	89	
Trend	2	AND	0.17	8.94	6.52	0.91	0.81	0.43	6.26	88	C1,C2
RCBD	1	SAL	0.15	5.96	5.53	0.86	0.76	0.38	6.94	80	
Trend	1	SAL	0.12	7.01	4.97	0.89	0.69	0.35	6.94	79	C1,C4
RCBD	2	SAL	0.11	6.96	4.86	0.87	0.67	0.34	6.90	89	
Trend	2	SAL	0.09	8.38	4.41	0.90	0.61	0.31	6.90	89	C1
RCBD	1	LAS	0.20	4.19	6.48	0.81	0.90	0.45	7.02	80	
Trend	1	LAS	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
RCBD	2	LAS	0.27	3.37	7.30	0.77	1.04	0.52	7.14	89	
Trend	2	LAS	0.21	4.38	6.49	0.82	0.92	0.47	7.14	89	C2
RCBD	1	ACR	0.14	8.97	5.58	0.84	0.61	0.31	6.82	160	
Trend	1	ACR	0.13	10.21	5.25	0.85	0.58	0.29	6.82	160	
RCBD	2	ACR	0.17	7.83	6.09	0.84	0.66	0.34	6.77	178	
Trend	2	ACR	0.14	9.36	5.57	0.86	0.61	0.31	6.77	178	

MR = model residual

FV = F-value for testing entry main effect

CV = coefficient of variation

LSD = least significant difference at  $\alpha = 0.05$

ASE = average standard error value for differences among entry means

SETM = GLS set mean

DF = average degrees of freedom computed from all pair-wise differences among entry means.

Effects = indicates trend effects fitted at a particular environment and set combination. C stands for column, no row effects were fitted. The number following the letter C indicates what degree polynomial was fitted.

n/a = not applicable, no trend effects were significant



Table 2. Spearman rank correlation coefficients (SRRC) between entry means at a given set and environment and across environments for each set obtained from fitting trend effects at individual environment and set combinations and ignoring trend effects at individual set and environment combinations.

Environment	Set	SRRC <sup>†</sup>
Andrews	1	0.9681
Andrews	2	0.9674
Salisbury	1	0.9666
Salisbury	2	0.9731
Laurel Springs	1	n/a <sup>‡</sup>
Laurel Springs	2	0.9529
Across environments	1	0.9881
Across environments	2	0.9843

†all SRRC differed significantly ( $P \leq 0.001$ ) from 0.  
<sup>‡</sup> no trend effects were fitted at the Laurel Springs set 1 environment

Table 3. Analysis of variance (ANOVA) tables for the combined analysis across environments for GLS for each set.

Combined ANOVA for set 1 utilizing entry means from each environment obtained from a RCBD

Source	DF	SS	MS	Fvalue	Prob
entry <sup>†</sup>	80	103.909	1.299	8.970	<.000000001
entry x env <sup>†</sup>	160	23.167	0.145	1.506	0.002053500
pooled error	240	23.083	0.096		

Combined ANOVA for set 1 utilizing entry means from each environment obtained from fitting trend effects

Source	DF	SS	MS	Fvalue	Prob
entry	80	104.441	1.306	10.215	<.000000001
entry x env	160	20.449	0.128	1.538	0.001281119
pooled error	238	19.773	0.083		

Combined ANOVA for set 2 utilizing entry means from each environment obtained from a RCBD

Source	DF	SS	MS	Fvalue	Prob
entry	89	118.486	1.331	7.827	<.000000001
entry x env	178	30.275	0.170	1.557	0.000525567
pooled error	267	29.166	0.109		

Combined ANOVA for set 2 utilizing entry means from each environment obtained from fitting trend effects

Source	DF	SS	MS	Fvalue	Prob
entry	89	118.277	1.329	9.363	<.000000001
entry x env	178	25.265	0.142	1.797	0.000007231
pooled error	266	21.013	0.079		

<sup>†</sup>Entry and entry x environment interaction terms are from a combined analysis across environments utilizing entry means obtained at each environment. Entry was tested against the entry x env interaction variance, and entry x env was tested against the corresponding pooled error term.

Table 4. Entry means across environments for GLS rating.

Set	Entry	GLS	Set	Entry	GLS
1	1952-T10†	6.52	2	2019-T10	7.25
1	1954-T10	7.51	2	2021-T10	5.96
1	1955-T10	6.48	2	2022-T10	7.32
1	1956-T10	7.17	2	2023-T10	7.37
1	1957-T10	7.07	2	2024-T10	5.39
1	1958-T10	6.75	2	2025-T10	7.18
1	1959-T10	7.12	2	2026-T10	7.03
1	1960-T10	7.45	2	2027-T10	6.97
1	1953-T10	6.49	2	2020-T10	6.69
1	1961-T10	6.80	2	2028-T10	6.89
1	1962-T10	7.02	2	2029-T10	6.89
1	1963-T10	7.35	2	2030-T10	7.06
1	1964-T10	6.73	2	2031-T10	7.11
1	1965-T10	7.51	2	2032-T10	7.26
1	1966-T10	7.19	2	2033-T10	6.98
1	1967-T10	6.65	2	2034-T10	6.20
1	1968-T10	6.39	2	2035-T10	6.52
1	1969-T10	7.06	2	2036-T10	7.34
1	1970-T10	7.32	2	2037-T10	6.03
1	1971-T10	7.19	2	2038-T10	5.92
1	1972-T10	7.58	2	2039-T10	5.67
1	1973-T10	7.34	2	2040-T10	6.67
1	1974-T10	6.68	2	2041-T10	6.11
1	1975-T10	6.92	2	2042-T10	7.43
1	1976-T10	7.43	2	2043-T10	7.46
1	1977-T10	7.49	2	2044-T10	6.81
1	1978-T10	6.51	2	2045-T10	6.46
1	1979-T10	6.14	2	2046-T10	7.58
1	1980-T10	7.46	2	2047-T10	7.44
1	1981-T10	6.04	2	2048-T10	5.90
1	1982-T10	6.64	2	2049-T10	5.79
1	1983-T10	6.08	2	2050-T10	7.41
1	1984-T10	6.38	2	2051-T10	6.94
1	1985-T10	6.71	2	2052-T10	7.21
1	1986-T10	7.63	2	2053-T10	7.29
1	1987-T10	6.44	2	2054-T10	7.38
1	1988-T10	7.59	2	2055-T10	7.44
1	1989-T10	6.68	2	2056-T10	7.45
1	1990-T10	7.24	2	2057-T10	6.58
1	1991-T10	7.83	2	2058-T10	5.70
1	1992-T10	6.76	2	2059-T10	7.49
1	1993-T10	7.38	2	2060-T10	5.59
1	1994-T10	6.66	2	2061-T10	6.33

Set	Entry	GLS	Set	Entry	GLS
1	1995-T10	7.07	2	2062-T10	6.48
1	1996-T10	7.60	2	2063-T10	6.96
1	1997-T10	7.13	2	2064-T10	7.14
1	1998-T10	5.98	2	2070-T10	7.55
1	1999-T10	7.27	2	2071-T10	6.98
1	2000-T10	7.09	2	2072-T10	6.54
1	2001-T10	6.81	2	2073-T10	7.03
1	2002-T10	6.67	2	2074-T10	6.50
1	2003-T10	6.64	2	2075-T10	6.40
1	2004-T10	6.34	2	2076-T10	7.00
1	2005-T10	7.44	2	2077-T10	7.55
1	2006-T10	7.55	2	2078-T10	7.62
1	2007-T10	7.49	2	2079-T10	6.50
1	2008-T10	6.84	2	2080-T10	7.37
1	2009-T10	6.48	2	2081-T10	6.30
1	2010-T10	7.16	2	2082-T10	6.78
1	2011-T10	7.15	2	2083-T10	6.86
1	2012-T10	7.66	2	2084-T10	7.01
1	2013-T10	7.18	2	2085-T10	6.82
1	2014-T10	7.51	2	2086-T10	7.07
1	2015-T10	7.00	2	2087-T10	6.96
1	2016-T10	6.20	2	2088-T10	6.72
1	2017-T10	7.52	2	2089-T10	7.47
1	2018-T10	6.92	2	2090-T10	7.37
1	B104.T10	5.35	2	2091-T10	7.39
1	DK689	6.20	2	2092-T10	7.17
1	DK697	6.31	2	2093-T10	6.89
1	DK743	6.17	2	2094-T10	7.11
1	G8288	5.41	2	2095-T10	7.48
1	LH132.51	5.35	2	2096-T10	7.81
1	LH195.256	6.38	2	2097-T10	7.32
1	LH200.262	5.97	2	2098-T10	7.58
1	NC300.T10	7.75	2	2099-T10	6.76
1	NK91-R9	5.86	2	B104.T10	5.75
1	P31G98	6.53	2	DK689	6.26
1	P32K61	6.83	2	DK697	6.44
1	P3394	4.06	2	DK743	6.31
1	HC33.TR7322	5.81	2	G8288	5.26
	Exp mean	6.82	2	LH132.51	5.56
	RILT mean	6.99	2	LH195.256	5.87
	Check mean	5.91	2	LH200.262	6.34
	Parent mean	6.55	2	NC300.T10	7.70
	LSD 0.05 experiment	0.58	2	P31G98	6.55
	LSD 0.05 vs. checkmean	0.42	2	NK91-R9	6.07
	LSD 0.05vs.parent mean	0.50	2	P32K61	6.75

2	P3394	4.25
2	HC33.TR7322	5.96
Exp mean		6.77
RILT mean		6.89
Check mean		5.97
Parent mean		6.73
LSD .05 experiment		0.61
LSD .05 vs. check mean		0.45
LSD .05 vs. parent mean		0.53

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GLS = Gray leaf Spot, recorded on a one to nine scale with a one designated as susceptible and a nine as resistant.

† T10 stands for FR615xFR697

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Table 5. Entry means for DTP obtained from the combined analysis across environments.

Set	Entry	DTP <sup>†</sup>	Set	Entry	DTP
1	1952-T10 <sup>‡</sup>	76.47	2	2019-T10	74.06
1	1953-T10	76.72	2	2020-T10	76.06
1	1954-T10	74.64	2	2021-T10	75.83
1	1955-T10	75.13	2	2022-T10	74.62
1	1956-T10	75.82	2	2023-T10	75.52
1	1957-T10	75.59	2	2024-T10	74.95
1	1958-T10	75.31	2	2025-T10	76.17
1	1959-T10	75.34	2	2026-T10	73.76
1	1960-T10	77.72	2	2027-T10	74.73
1	1961-T10	76.03	2	2028-T10	75.71
1	1962-T10	75.00	2	2029-T10	77.72
1	1963-T10	75.45	2	2030-T10	74.84
1	1964-T10	76.03	2	2031-T10	75.11
1	1965-T10	77.22	2	2032-T10	77.45
1	1966-T10	76.04	2	2033-T10	75.44
1	1967-T10	74.37	2	2034-T10	74.72
1	1968-T10	75.27	2	2035-T10	74.18
1	1969-T10	75.30	2	2036-T10	74.99
1	1970-T10	75.19	2	2037-T10	75.11
1	1971-T10	74.73	2	2038-T10	74.24
1	1972-T10	77.32	2	2039-T10	74.10
1	1973-T10	74.76	2	2040-T10	74.25
1	1974-T10	77.64	2	2041-T10	74.81
1	1975-T10	75.69	2	2042-T10	76.84
1	1976-T10	76.06	2	2043-T10	75.60
1	1978-T10	75.85	2	2044-T10	74.95
1	1979-T10	74.98	2	2045-T10	73.95
1	1980-T10	74.99	2	2046-T10	76.10
1	1981-T10	75.15	2	2047-T10	75.35
1	1982-T10	74.91	2	2048-T10	74.58
1	1983-T10	74.60	2	2049-T10	75.11
1	1984-T10	74.15	2	2050-T10	76.31
1	1985-T10	74.99	2	2051-T10	77.09
1	1986-T10	77.25	2	2052-T10	74.98
1	1987-T10	74.69	2	2053-T10	75.87
1	1988-T10	75.94	2	2054-T10	74.53
1	1989-T10	73.62	2	2055-T10	76.58
1	1990-T10	74.32	2	2056-T10	75.61
1	1991-T10	74.24	2	2057-T10	76.49
1	1992-T10	74.88	2	2058-T10	74.53
1	1993-T10	77.54	2	2059-T10	74.64
1	1994-T10	74.27	2	2060-T10	74.69

Set	Entry	DTP
1	1995-T10	74.12
1	1996-T10	76.66
1	1997-T10	74.89
1	1998-T10	75.16
1	1999-T10	77.06
1	2000-T10	75.20
1	2001-T10	74.63
1	2002-T10	75.25
1	2003-T10	75.57
1	2004-T10	75.11
1	2005-T10	76.28
1	2006-T10	76.87
1	2007-T10	77.46
1	2008-T10	75.90
1	2009-T10	74.10
1	2011-T10	75.53
1	2012-T10	75.07
1	2013-T10	76.18
1	2014-T10	77.92
1	2015-T10	77.42
1	2016-T10	73.64
1	2017-T10	75.96
1	2018-T10	75.38
1	DK697	76.43
1	G8288	73.17
1	LH200.200	75.75
1	P31G98	76.16
1	P32K61	74.46
1	HC33TR722	72.73
Exp mean		75.51
RILT mean		75.58
Check mean		74.79
lsd 0.05Exp		1.13
lsd 0.05RILT vs. check mean		0.86

Set	Entry	DTP
2	2061-T10	75.56
2	2062-T10	76.01
2	2063-T10	77.45
2	2064-T10	76.45
2	2070-T10	76.93
2	2071-T10	75.20
2	2072-T10	75.81
2	2073-T10	74.03
2	2074-T10	75.38
2	2075-T10	74.57
2	2076-T10	75.18
2	2077-T10	76.91
2	2078-T10	76.65
2	2079-T10	74.03
2	2080-T10	76.01
2	2081-T10	76.01
2	2082-T10	76.62
2	2084-T10	75.06
2	2085-T10	74.14
2	2086-T10	74.47
2	2087-T10	74.35
2	2088-T10	74.75
2	2089-T10	76.47
2	2090-T10	75.44
2	2091-T10	75.49
2	2092-T10	74.04
2	2093-T10	75.48
2	2094-T10	76.49
2	2095-T10	74.22
2	2096-T10	77.22
2	2097-T10	75.36
2	2098-T10	75.73
2	2099-T10	74.88
2	DK697	74.89
2	G8288	73.47
2	LH200.200	76.38
2	P31G98	76.15
2	P32K61	75.65
2	HC33TR7322	72.80
Exp mean		75.36
RILT mean		75.39
Check mean		74.89
lsd 0.05	Exp	1.32

lsd 0.05 RILT vs. check mean 1.01

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†DTP = days to fifty-percent pollen shed.

‡T10 = T10 stands for FR615xFR697.

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Table 6. Estimates of genetic parameters and their corresponding standard errors (SE).

Heritability estimates on a plot and entry mean basis for GLS and DTP

Trait	Type	H	SE <sup>†</sup>
GLS	PLOT	0.4648	0.0407
GLS	ENTRY	0.8157	0.0270
DTP	PLOT	0.3390	0.0370
DTP	ENTRY	0.7896	0.0293

Genetic and phenotypic correlations between GLS and DTP

Type	Correlation	SE
Genetic	0.4557	0.1249
Phenotypic	0.3297	0.0434

†: SE were calculated using the delta method

Table 7. Summary of GLS QTL positions,  $R^2$ , effects, and logarithm of the odds (LOD) scores from the analysis of a given set and environment and across environments obtained from fitting trend effects at individual environments (TREND) and ignoring trend effects (RCBD).

PDS	Chromosome	RCBD				Trend			
		Position <sup>†</sup>	$R^2$	Effect <sup>‡</sup>	LOD	Position <sup>†</sup>	$R^2$	Effect <sup>‡</sup>	LOD
OVE	1	42.01	0.154	0.205	2.26	48.01	0.124	0.183	2.20
	4	111.85	0.270	0.278	8.76*	113.85	0.245	0.264	8.79*
	8	48.43	0.083	0.154	2.73	42.43	0.068	0.138	2.84
AND	1	40.01	0.029	0.154	1.79	61.53	0.029	0.128	1.29
	4	88.69	0.105	0.256	3.90*	88.69	0.108	0.250	3.97*
	4	119.25	0.081	0.232	3.17*	117.25	0.070	0.214	3.34*
SAL	8	48.43	0.069	0.189	2.47	46.43	0.064	0.177	2.59
	1	73.53	0.057	0.132	1.31	73.53	0.058	0.127	1.40
	4	111.85	0.157	0.220	4.87*	111.85	0.220	0.254	6.90*
LAS	8	52.43	0.124	0.203	3.43*	48.43	0.134	0.199	3.99*
	1	38.01	0.295	0.301	3.98*	46.01	0.239	0.273	3.67*
	4	115.85	0.063	0.142	2.13	115.85	0.061	0.141	2.09
	8	81.43	0.104	0.180	2.45	75.43	0.138	0.209	2.76

PDS = Phenotypic data set

OVE= combined analysis across environments

AND= Andrews environment

SAL=Salisbury environment

LAS= Laurel Springs environment

<sup>†</sup> Positions reported have the highest LOD score at a given chromosomal region and are reported in centi-Morgans for the corresponding chromosome

<sup>‡</sup> Effects are expressed relative to NC300, i.e. the sign of the effect corresponds to the magnitude of the NC300 allele

\* Indicates significance for the corresponding phenotypic data set's threshold

Table 8. Summary of DTP QTL positions,  $R^2$ , effects, and logarithm of the odds (LOD) scores from the analysis at a given set and environment and across environments obtained from fitting trend effects at individual environments (TREND) and ignoring trend effects (RCBD).

PDS	Chrom	RCBD				Trend			
		Pos <sup>†</sup>	$R^2$	Effect <sup>‡</sup>	LOD	Pos	$R^2$	Effect	LOD
OVE	1	208.0	0.14	0.40	4.60*	210.0	0.17	0.43	5.34*
	4	179.0	0.17	0.44	6.54*	179.0	0.18	0.44	7.01*
	5	178.0	0.12	-0.37	2.42	122.0	0.16	-0.40	3.01*
	8	111.0	0.11	0.34	4.13*	111.0	0.09	0.31	3.56*
AND	1	208.0	0.07	0.32	1.98	210.0	0.08	0.32	2.44
	4	181.0	0.12	0.41	4.16*	179.0	0.12	0.38	4.34*
	5	124.0	0.14	-0.45	2.97*	126.0	0.20	-0.49	4.51*
	8	129.0	0.07	0.32	1.61	115.0	0.06	0.28	2.39
LAS	1	210.0	0.11	0.55	2.78*	206.0	0.08	0.45	2.50
	4	181.0	0.03	0.30	1.13	183.0	0.04	0.32	1.67
	5	120.0	0.06	-0.40	0.91	120.0	0.07	-0.42	1.31
	8	106.0	0.12	0.55	3.58*	106.0	0.12	0.53	4.13*
C02	1	208.0	0.15	0.49	4.67*	210.0	0.18	0.53	5.23*
	4	181.0	0.15	0.49	5.88*	179.0	0.18	0.54	6.64*
	5	170.0	0.11	-0.42	3.36*	170.0	0.12	-0.44	3.85*
	8	113.0	0.02	0.20	0.91	109.0	0.02	0.17	0.77
C03	1	208.0	0.07	0.32	1.98	208.0	0.10	0.40	2.75
	4	183.0	0.11	0.39	4.19*	179.0	0.11	0.40	3.23*
	5	124.0	0.14	-0.45	2.97	132.0	0.05	-0.28	1.23
	8	129.0	0.07	0.32	1.61	107.0	0.06	0.29	2.19

PDS = Phenotypic data set

OVE= combined analysis across environments

AND= Andrews environment

LAS= Laurel Springs environment

C02= Clayton 2002 environment

C03= Clayton 2003 environment

Chrom =chromosome

† Positions reported have the highest LOD score at a given chromosomal region and are reported in centi-Morgans for the corresponding chromosome

‡ Effects are expressed relative to NC300, i.e. the sign of the effect corresponds to the magnitude of the NC300 allele

\* Indicates significance for the corresponding phenotypic data set's threshold

Table 9 (a) and (b). Summary of the epistatic interaction between marker bnlgl175 and umc2182. (a) Displays marker genotypes, sample size for each of the four different genotype by genotype interactions and their respective mean GLS rating and standard errors. (b) Displays the additive main effects and corresponding standard errors for both marker loci and also the additive effect at marker bnlgl175 given a NC300 genotype at marker umc2182, the additive effect at marker bnlgl175 given a B104 genotype at marker umc2182, the additive effect at marker umc2182 given a NC300 genotype at marker bnlgl175, the additive effect at marker umc2182 given a B104 genotype at marker bnlgl175.

(a)

Bnlgl175	Umc2182	N	GLS	SE
NC300	NC300	28	6.94	0.09
NC300	B104	37	7.10	0.09
B104	NC300	30	7.05	0.08
B104	B104	34	6.53	0.08

(b)

Source	Effect <sup>†</sup>	S.E.	Prob
Additive effect for Bnlgl175	0.11	0.04	0.0066
Additive effect for Umc2182	0.09	0.04	0.0250
Additive effect for Bnlgl175 for BB <sup>‡</sup>	-0.06	0.06	0.3421
Additive effect for Bnlgl175 for bb	0.28	0.06	0.0001
Additive effect for Umc2182 for AA	-0.08	0.06	0.2168
Additive effect for Umc2182 for aa	0.26	0.06	0.0001

Bnlgl175 = SSR marker locus

Umc2182 = SSR marker locus

N = sample size

GLS = Gray leaf spot rating.

S.E. = Standard error.

Source = specifies the appropriate effect

<sup>†</sup> Effect = Half the deviation between NC300 and B104 genotypic values at a particular locus and are expressed relative to NC300.

<sup>‡</sup> AA designates the NC300 genotype at marker Bnlgl175, aa designates the B104 genotype at Bnlgl175. BB designates NC300 genotype at marker Umc2182, and bb designates B104 genotype at marker Umc2182

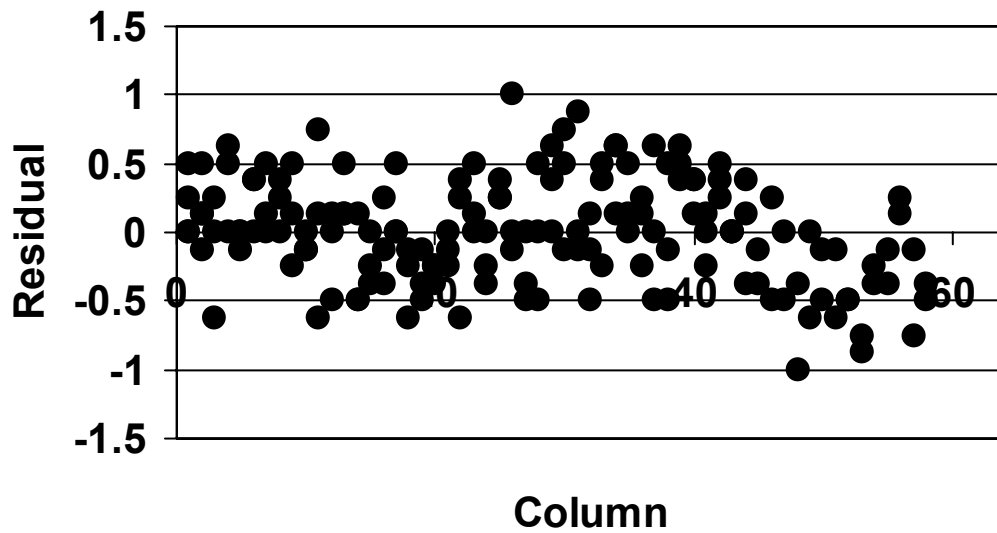
Table 10. Multi-locus ANOVA table for GLS rating.

Source	Chromosome	DF	Type III SS	Mean Square	F	Prob
bnlg1621	4	1	3.3040	3.3040	18.21	<.0001
bnlg147	1	1	1.2964	1.2964	7.15	0.0082
bnlg1175	2	1	0.4053	0.4053	2.23	0.1383
umc2182	8	1	0.8158	0.8158	4.50	0.0366
bnlg1175*umc2182		1	2.3562	2.3562	12.99	0.0005

$R^2 = 0.35$ ,  $N = 121$ .

Figure 1. Residual versus column plots for GLS in set 1 and 2 at the Andrews environment.

### Andrews Set 1 Column Trend



### Andrew Set 2 Column Trend

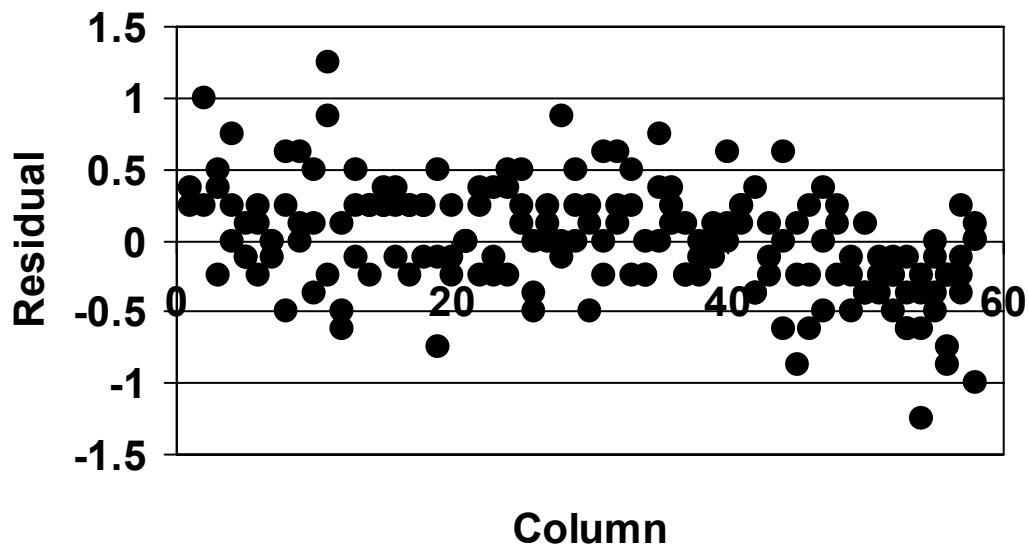


Figure 2. Residual versus column plots for GLS in set 1 and 2 at the Salisbury environment.

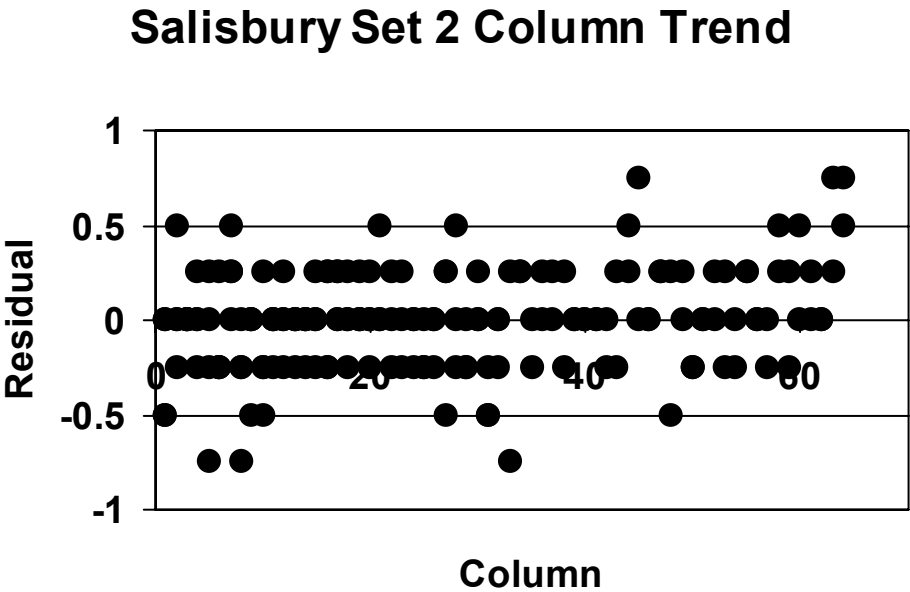
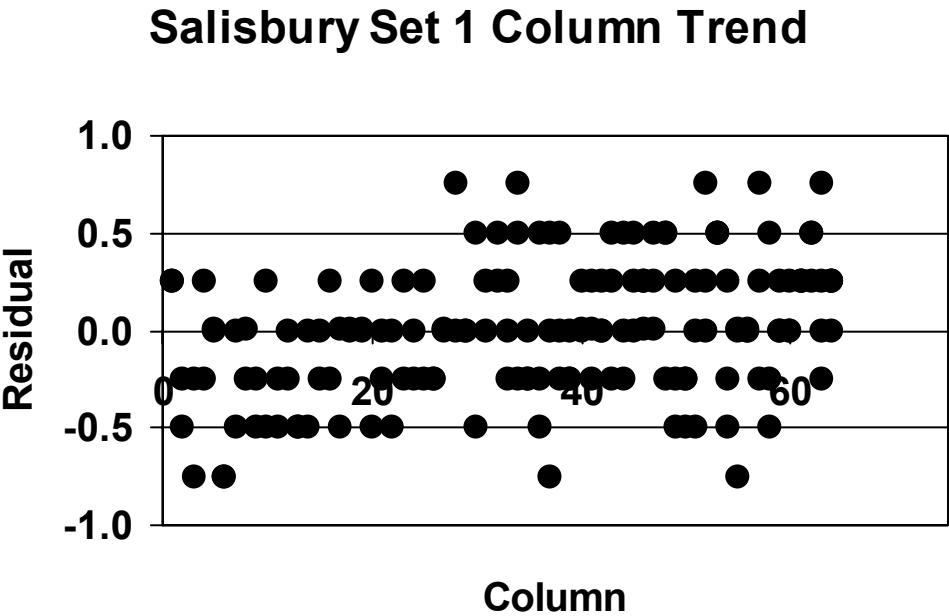
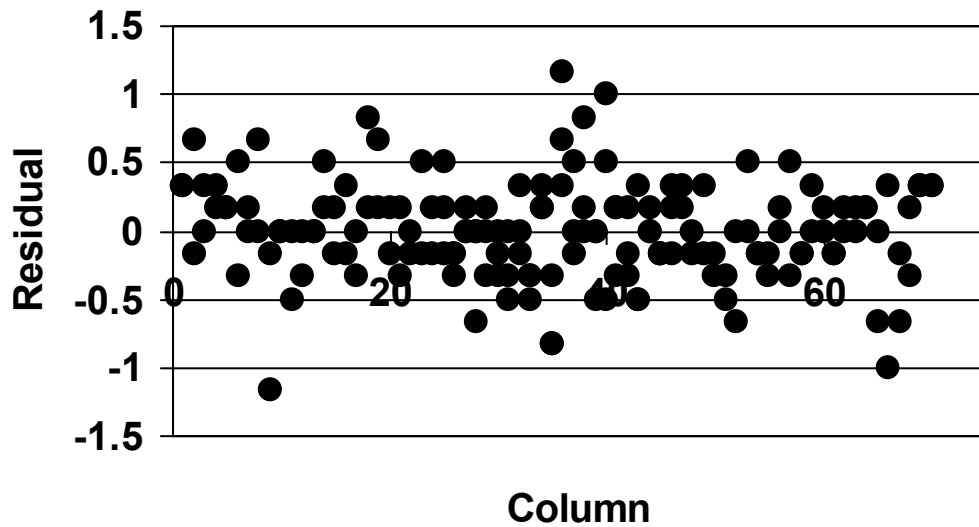
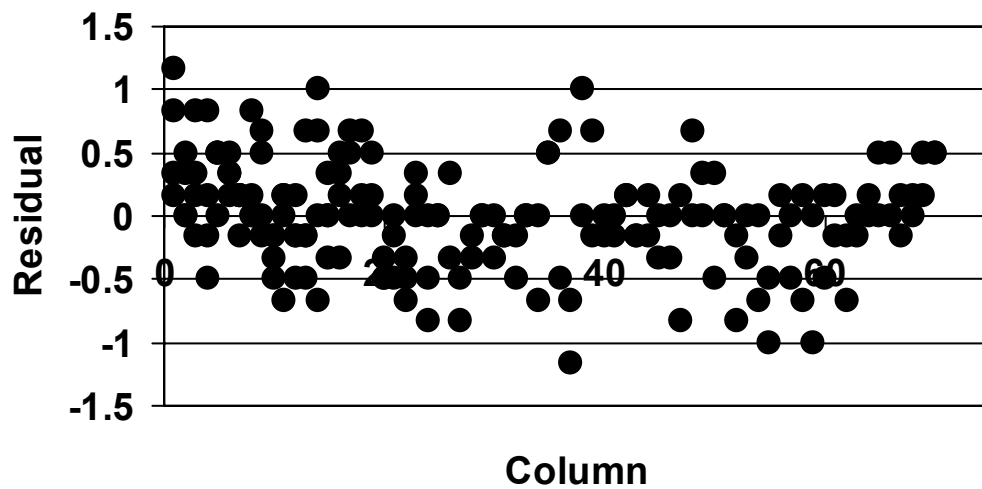


Figure 3. Residual versus column plots for GLS in set 1 and 2 at the Laurel Springs environment.

### Laurel Springs Set 1 Column Trends



### Laurel Springs Set 2 Column Trends





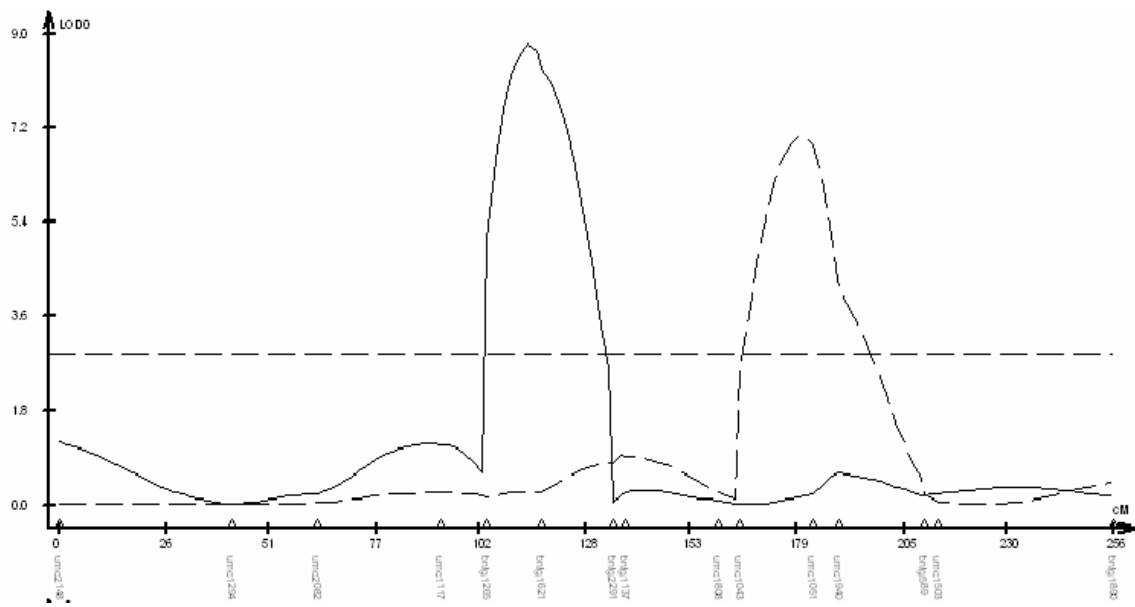


Figure 4. LOD profile map of QTL effects on GLS rating and DTP for chromosome 4 from the combined analysis where trend effects were fitted at individual environments for each trait. Solid line indicates the LOD profile for GLS rating. Dashed line indicates the LOD profile for DTP. Horizontal dashed line indicates the significance threshold for DTP.

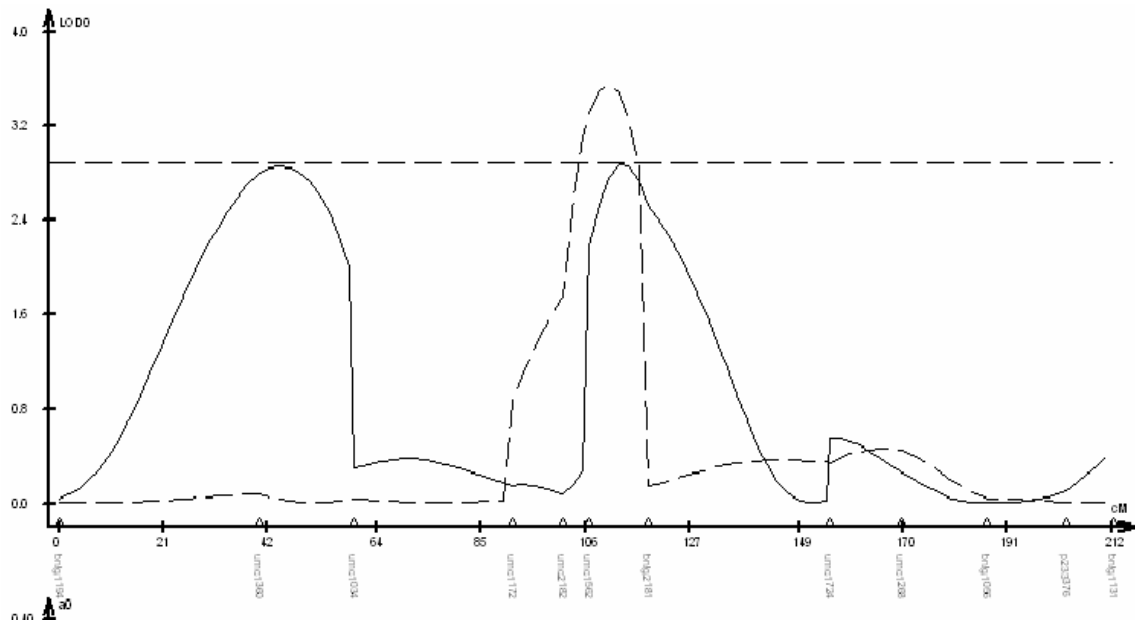


Figure 5. LOD profile map of QTL effects on GLS rating and DTP for chromosome 8 from the combined analysis where trend effects were fitted at individual environments for each trait. Solid line indicates the LOD profile for GLS rating. Dashed line indicates the LOD profile for DTP. Horizontal dashed line indicates the significance threshold for DTP. Note the GLS rating profile did not exceed the threshold. Further, the first peak for GLS rating corresponds to SALGLSQT1.

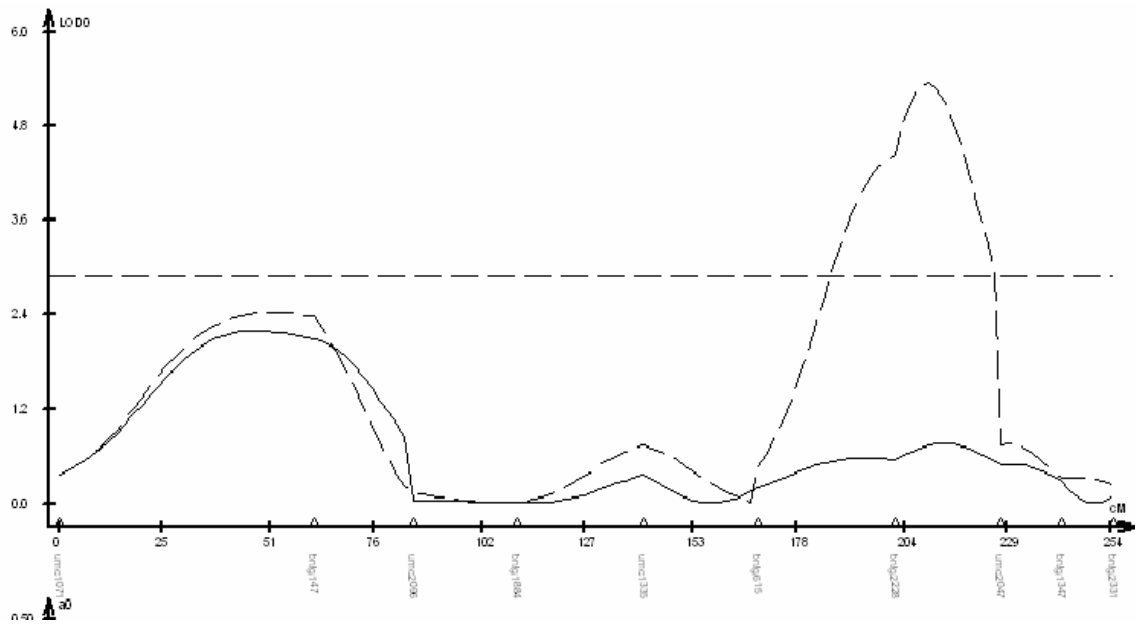


Figure 6. LOD profile map of QTL effects on GLS rating and DTP for chromosome 1 from the combined analysis where trend effects were fitted at individual environments for each trait. Solid line indicates the LOD profile for GLS rating. Dashed line indicates the LOD profile for DTP. Horizontal dashed line indicates the significance threshold for DTP. Note, the GLS rating profile did not exceed the threshold. Further, the first peak for GLS rating corresponds to LASGLSQTL1.

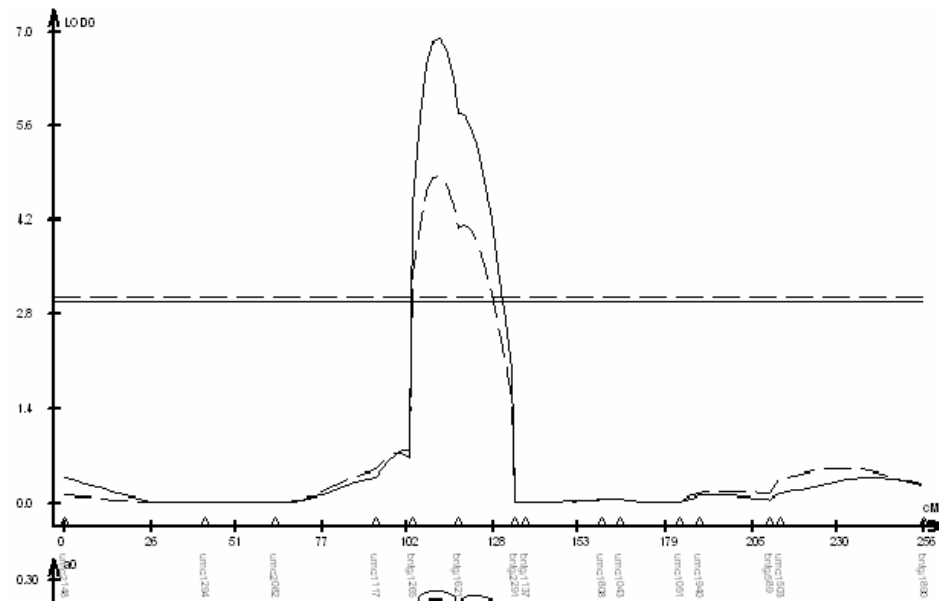


Figure 7. LOD profile map of QTL effects on GLS rating for chromosome 4 from analyses at Salisbury. Solid line indicates the LOD profile for GLS rating obtained from adjusting entry means according to trend effects. Dashed line indicates the LOD profile for GLS rating performed on entry means using a RCBD. Horizontal solid and dashed lines indicate the significance threshold from the trend and RCBD, respectively.

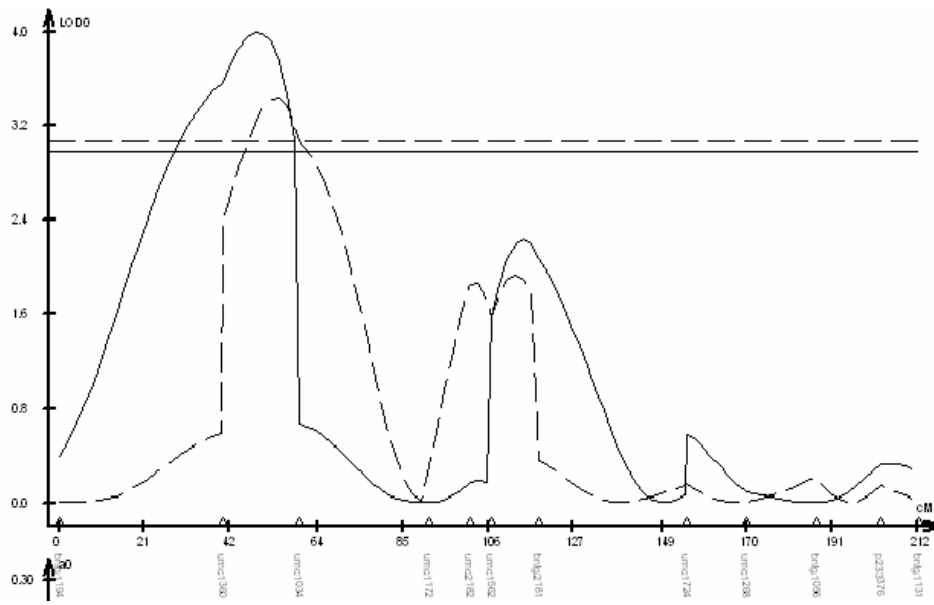


Figure 8. LOD profile map of QTL effects on GLS rating for chromosome 8 from analyses at Salisbury. Solid line indicates the LOD profile for GLS rating obtained from adjusting entry means according to trend effects. Dashed line indicates the LOD profile for GLS rating performed on entry means using a RCBD. Horizontal solid and dashed lines indicate the significance threshold from the trend and RCBD, respectively.

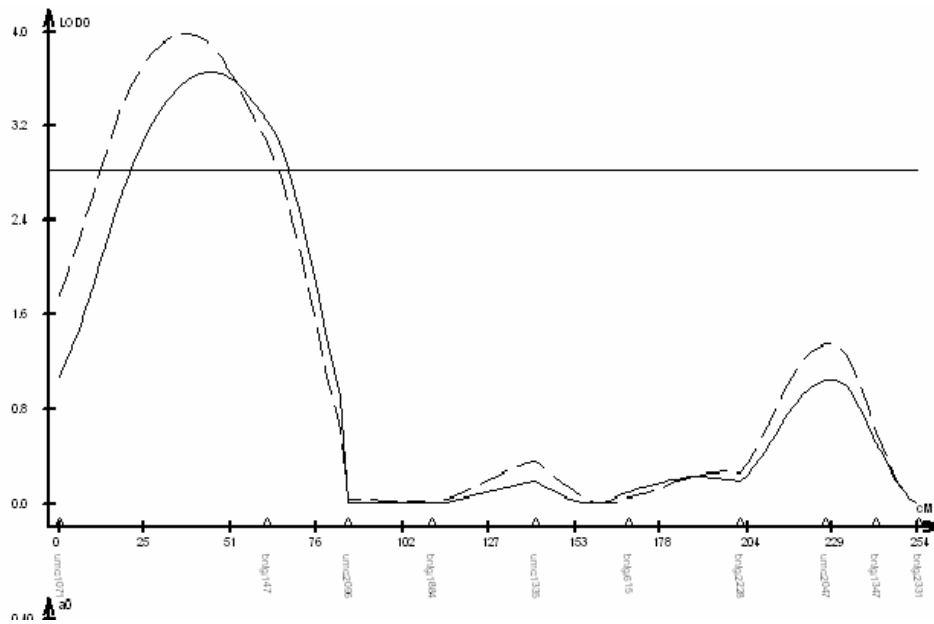


Figure 9. LOD profile map of QTL effects on GLS rating for chromosome 1 from analyses at Laurel Springs. Solid line indicates the LOD profile for GLS rating obtained from adjusting entry means according to trend effects. Dashed line indicates the LOD profile for GLS rating performed on entry means using a RCBD. Horizontal solid and dashed lines indicate the significance threshold from the trend and RCBD, respectively.

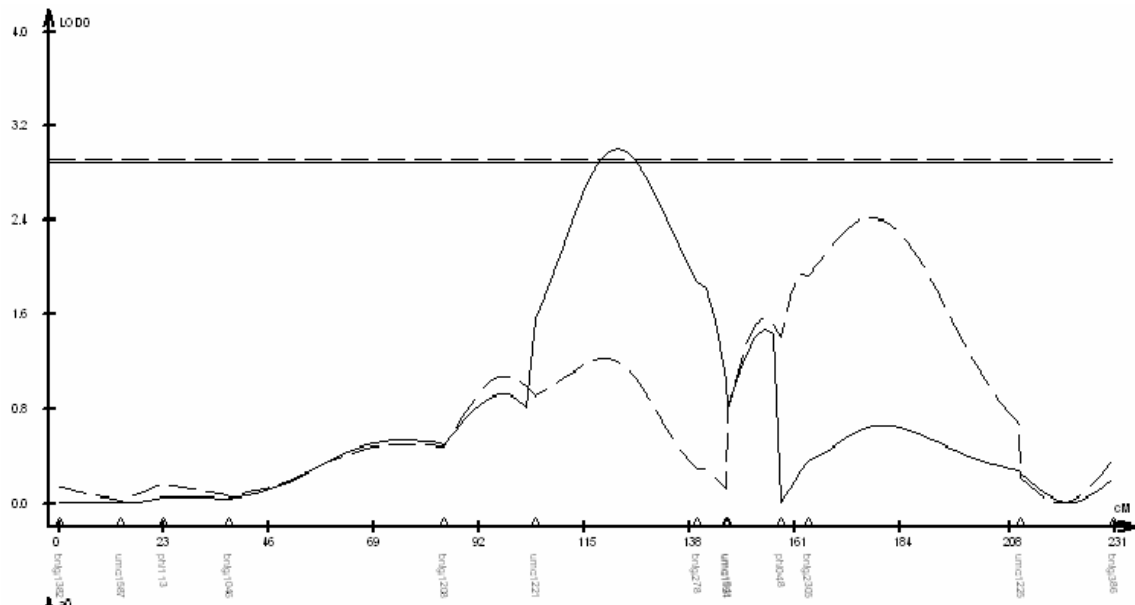


Figure 10. LOD profile map of QTL effects on DTP for chromosome 5 from the two combined analyses across environments. Solid line indicates the LOD profile for DTP obtained from combined analysis where trend effects were fitted at individual environments. Dashed line indicates the LOD profile for DTP obtained from combined analysis where trend effects were ignored. Horizontal solid and dashed lines indicate the significance threshold from fitting trend effects and not fitting trend effects, respectively.

### **III. Agronomic Evaluation and Identification of Quantitative Trait Loci (QTL) Associated with Grain Yield in a Semi-Tropical Recombinant Inbred Population of Maize.**



## Abstract

Tropical germplasm in maize (*Zea mays* L.) is a valuable resource to help decrease the dependence upon a limited genetic base currently used to produce commercial hybrids in the U.S. corn belt and extend selection limits for grain yield. Experiments were conducted to evaluate 143 S<sub>4,5</sub> recombinant inbred lines (RILs) resulting from a cross between NC300, an all-tropical, temperate adapted line, and B104, a stiff stalk line. The 143 RILs were topcrossed to the Lancaster tester FR615xFR697 and randomly subdivided into two sets. The two sets were evaluated in yield trials in 2002 and 2003 in a total of eight North Carolina environments. Twenty-one RILs from both sets did not differ significantly ( $P = 0.05$ ) for grain yield when compared to the mean of the commercial checks. One RIL, RIL 2070, yielded significantly ( $P = 0.05$ ) higher than the lowest yielding commercial check, HC33.TR7322. The twenty-one RILs also were within the range for the commercial checks for other agronomic traits such as percent moisture, percent erect plants, ear height, plant height, days to fifty-percent pollen shed, days to mid-silk, and anthesis-silk interval. The RILs were genotyped at 94 simple sequence repeats (SSRs) loci. Composite interval mapping (CIM) was performed on the RILT for grain yield on a combined analysis across all environments, across environments within a given year, and at individual environments. A QTL was identified on chromosome 1 from the combined analysis across the 2003 environments. The NC300 allele had an increasing effect of 0.15 t ha<sup>-1</sup> for the QTL on chromosome 1 and explained 11.0% of the phenotypic variation. A QTL was also detected on chromosome 3 from the combined analysis across 2002 and 2003 environments. The B104 allele had an increasing effect of 0.20 t ha<sup>-1</sup> on chromosome 3 and explained 31.0% of the phenotypic

variation. B104 also had alleles associated with an increasing effect for other grain yield QTLs identified on chromosomes 7 and 9. Significant markers identified from CIM were subjected to a multi-factor Analysis of Variance (ANOVA) to test for marker-by-environment interaction. All markers, except the marker corresponding to the QTL on chromosome 3 displayed significant ( $P \leq 0.01$ ) marker-by-environment interaction. These experiments demonstrated that inbred lines possessing a large amount of tropical germplasm can be competitive with commercial hybrids for grain yield and other agronomic traits. Further, both parents contributed favorable alleles to the RILT for grain yield.

## Introduction

The germplasm base for maize (*Zea Mays* L.) grown in the U.S. is based upon the heavy recycling of eight inbred lines and their derivatives (Goodman 1992). These inbred lines include A632, B14A, B37, B73, B84, C103, Mo17, and Oh43. All of these lines are derived from one race of corn, Corn Belt Dent. The Corn Belt Dent race was developed from the intercrossing of two other racial complexes (Anderson and Brown, 1952), the southern dents and the northern flints, and is only one of around 250 known races of maize (Goodman and Brown, 1988; Troyer, 1999). Efforts suggesting the importance of utilizing alternative methods to broaden the U.S. maize germplasm base have been quite extensive (Wellhausen, 1956; Hallauer, 1978; Geadelmann, 1984; Goodman, 1985; Bridges and Gardner, 1987). Despite these efforts, the incorporation of exotic germplasm into hybrids grown in the U.S. Corn Belt has almost been non-existent. Goodman (1998) estimated the percentage of exotic germplasm in hybrids grown in U.S. fields in 1996 to be around 1.0%. Simmonds (1993) defined incorporation as, “the systematic exploitation of a large array of genetic variability in such a way as to generate a mass of newly adapted stocks usable as parents in breeding programs, versus introgression, as backcrossing into adapted stocks of a few genes controlling desired characters.”

Hallauer (1988) defined exotic germplasm as “all germplasm that does not have immediate usefulness without selection for adaptation to a given area.” U.S. maize breeders can consider exotic germplasm to include tropical sources, temperate sources that are foreign such as inbred lines F2 and EP1, and also domestic temperate sources, such as landraces. Explanations for minimal incorporation of exotic germplasm into

temperate U.S. commercial maize breeding programs are numerous. These explanations are centered on investment in time and money, uncertainty of deriving a useful product, which all lead to a lack of funding or emphasis for such work.

Specifically, tropical materials are sensitive to long day photoperiods, susceptible to common smut (*Ustilago maydis*), have weak stalks, poor silk and tassel synchronization, and poor inbred *per se* performance (Goodman, 1985). These biological barriers that U.S. commercial maize breeders must overcome require time and are further complicated by the gap between breeding improvements in the tropics and elite temperate materials found throughout the U.S. (Goodman 1985). In addition, while genetic variation is usually large in an adapted by tropical population, the mean yield is generally low.

Advanced cycle breeding has allowed for the continued improvement in hybrid maize at a rate of 125.4 kg ha<sup>-1</sup> per year (Duvick, 1992). Questions have arisen concerning the sustainability of such a rate of increase in yield. Selection limits are primarily influenced by genetic variance (Falconer and Mackay, 1996). U.S. maize breeders rely upon two heterotic groups from the Corn Belt Dent race to develop improved single-crosses, the stiff-stalks (BSSS) and non-stiff stalks groups (Hallauer and Miranda, 1988). Lu and Bernardo (2001) used principal component analysis on SSR data to cluster maize inbred lines to obtain average genetic distances for various sets of lines. They found the average genetic distance among a set of elite BSSS and non-stiff stalk lines was high compared to the average genetic distances for elite lines within each of the groups. Lu and Bernardo (2001) suggested that the reduction of the average genetic

distance among elite lines within a heterotic group could limit future gains from selection.

Tropical germplasm could allow for greater long term progress to selection for yield through widening the germplasm base of maize (Hallauer 1978; Stuber 1978; Goodman 1985). Also, tropical materials could help reduce genetic uniformity, which has the potential to translate into genetic vulnerability (Committee on Genetic Vulnerability, 1972).

The question arises as to what type of source population and breeding scheme to choose to initiate selection upon. In terms of breeding strategies, several have promoted mass selection and recurrent selection schemes to adapt and improve broad based populations (Bridges and Garnder, 1987; Genter 1976; Hallauer and Sears, 1972; Hallauer, 1978). Theoretical studies have suggested backcrossing the exotic material to an improved source before initiating selection (Bailey and Comstock, 1976; Cox., 1984; Dudley, 1982 and 1984; Ho and Comstock 1980 ). Backcrossing objectives, however, usually entail improving a line or population by only a few factors. Goodman (1992) suggested using tropical hybrids, whose parents have already undergone inbreeding as sources for new inbred line development.

Several studies demonstrated that topcrosses between 100% tropical lines and a U.S. tester were competitive with commercial checks (Uhr and Goodman, 1995; Holley and Goodman, 1988). Holland and Goodman (1995) showed that various racial accessions that went through a photoperiodic conversion following crossing to Mo44, displayed resistance to diseases such as Gray Leaf Spot (GLS)[*Cercospora zea-maydis* Tehon and Daniels]. Goodman (1999) identified crosses between commercial hybrids

and NC296A that out-yielded the commercial hybrids themselves by an average of about 1 t ha<sup>-1</sup> at a cost of 1.6% grain moisture and 8% lower erect plants. Kraja and Dudley (2000), using Dudley's method for identifying populations with favorable alleles, identified several accessions from the Germplasm Enhancement of Maize Project that possessed favorable dominant alleles for resistance to common rust [*Puccinia sorghi* Schwein], Gray Leaf Spot [*Cercospora zea-maydis* Tehon and Daniels], and Southern Corn Leaf Blight [*Bipolaris maydis* (Nisikado & Miyake) Shoemaker = *Helminthosporium maydis* Nisikado & Miyake] that were not present in FR1064 x LH185, a dominant hybrid marketed throughout the corn belt in the late 1990's.

Semi-tropical recombinant inbred lines (RILs) were developed from a cross between NC300, an all-tropical line, and B104, a stiff stalk line. The RILs were topcrossed to a sister-line tester, FR615xFR697, to first assess if any of the RILT were within the range of the commercial checks for grain yield. Secondly, we wanted to compare favorable RILT to commercial checks for agronomic traits such as plant height, maturity, root and stalk lodging, and grain moisture at harvest. Several papers have demonstrated that exotic lines or populations possess favorable alleles at genomic regions for grain yield (Ragot M. et al., 1995; and Kozumplik et al., 1996). A third objective of this study was to identify QTL associated with grain yield and to assess if NC300 possesses alleles at identified QTL that are associated with increased grain yield.

## **Materials and Methods**

### **Development of the Population**

A semi-tropical recombinant inbred population was developed from the cross between NC300 and B104. It was initiated by Dr. Terry Molnar and was provided by Dr. Major M. Goodman at North Carolina State University. NC300, an all-tropical, temperate-adapted line, is a second-cycle line derived from a cross between two experimental lines derived from hybrids PioneerX105A and PioneerX306B x H5, respectively. Pioneer X105A and Pioneer X306B are two double cross tropical hybrids developed in Jamaica during the 1970's. Pioneer X105A consists of Cuban Flint, ETO, and Tuxpeño germplasm, and Pioneer X306B possesses Chandelle, Tusón, Coastal Tropical Flint, and Cuban Flint germplasm. H5, a double-cross tropical hybrid, contains Cuban Flint and Tuxpeño germplasm and was developed by the CNTA program in El Salvador. For more information about inbred lines developed from these tropical hybrids, refer to Holley and Goodman (1988). B104 is a temperate line developed at Iowa State University and is derived from BS13(S)C5-13-1 (Hallauer et al., 1997). The BS13 is a strain of BSSS that has undergone 12 cycles of recurrent selection, primarily for grain yield.

The initial cross was generated in the winter nursery in 1998 in Homestead, Florida.  $F_1$  seed was planted the following spring in the greenhouse and was self-pollinated to produce  $S_0$  seed.  $S_0$  plants were grown in the Raleigh, 1999, summer nursery. After self pollinating 143  $S_0$  plants, seed from each  $S_0$  plant was used to plant one row in Homestead, Florida in 1999. The  $S_{0:1}$  rows were self-pollinated, and seed from one of the ears from each  $S_{0:1}$  row was sampled to plant an entire row in the next

generation. This process was repeated so that every  $S_0$  plant was represented by a descendent in the proceeding generations of inbreeding in an attempt to randomly derive inbred lines using a modified single-seed-descent approach. Each successive generation was subjected to this type of treatment until 143 randomly derived  $S_{4.5}$  recombinant inbred lines (RILS) were established with an inbreeding coefficient equivalent to  $F=0.96875$  (Cockerham, 1983). The 143 RILs were topcrossed (RILT) to the Lancaster tester FR615xFR697, a C103 sister line cross (MBS, 2001).

### **Experimental design**

Yield trials were conducted at four North Carolina locations, Clayton, Plymouth, Lewiston, and Jackson Springs in 2002 and 2003. The 143 RILT were assigned to one of two sets, and the experimental design deployed was a replication-within-sets design. Each set was replicated two times in 2002, and three times in 2003. Treatments, or RILT and commercial checks, are referred to as entries. Set 1 in 2002 consisted of seventy-two entries including sixty-five RILT and seven commercial hybrid checks. In 2003, set one consisted of eighty-one entries including sixty-seven RILT and twelve commercial hybrid checks. Set 2 in 2002 consisted of eighty-one entries including seventy-five RILT and six commercial hybrid checks. In 2003, set 2 consisted of ninety entries including seventy-six RILT and twelve commercial hybrid checks. Both sets in 2003 shared the same commercial checks, and each set included NC300 and B104 topcrosses to FR615xFR697.

Experimental units consisted of two-row, 44 plant plots, 4.86 m in length, at all locations. A 1-m alley was allocated at the end of each plot at all locations. Inter-row spacing was 0.97 m at Clayton, Plymouth, and Jackson Springs, and 0.91 m at Lewiston.



Plots were over-planted for a target plant density of 43,000 plants ha<sup>-1</sup> at Clayton, Plymouth, and Jackson Springs, and 45,000 plants ha<sup>-1</sup> at Lewiston. Plots were subjected to standard North Carolina cultural practices. The experimental design for set 1 at all locations in 2002 and 2003 was a 9 x 8 x 2 and a 9 x 9 x 3 lattice, respectively. For set 2, the experimental design at all locations in 2002 and 2003 was a 9 x 9 x 2 and 10 x 9 x 3 lattice, respectively.

Response variables measured on all yield-trial plots included grain yield (t ha<sup>-1</sup>) adjusted to 15.5% moisture, percentage grain moisture at harvest, percentage lodging, ear height (cm from the base of a single representative plant to the uppermost ear node), and plant height (cm from the base of a single representative plant to the tip of the tassel). Days to mid-silk and fifty-percent pollen shed were measured only at the Clayton location in both years. Anthesis-silk interval (ASI) was calculated by taking the difference between days to 50% pollen shed and days to mid-silk. A given location and year combination was considered a single environment.

### **Statistical analysis for phenotypic data**

In the analyses for all response variables, entries were treated as fixed effects, we were interested in this specific set of RILT. Common commercial checks among both sets and years were retained for adjusting the RILT for set effects. Entry means, for a given set and environment, were first obtained from an analysis of variance (ANOVA) utilizing the corresponding lattice design. Secondly, a trend analysis, or polynomial regression, was used according to Brownie et al., (1993). The model for the lattice design included the rep, and the residual variance, block nested within rep-by-entry interaction, as random effects.

The trend analysis and ANOVA utilizing the lattice design were compared to each other on the basis of precision. Precision was defined by the standard errors of differences among entry means. Therefore, standard errors were calculated for differences among all entry means for both analyses. Entry means obtained from the analysis that provided the smallest average standard error (ASE) value, for a given set and environment, were generated using the LSMEANS statement in PROC MIXED of SAS V 8.0 (SAS Institute, Cary, N.C.) and entry means from the superior analysis were included in further analyses.

Fisher's protected LSD (Steel et al., 1997) was used for entry mean separation for all response variables; entries in the same set were analyzed separate from entries in the other set. An ANOVA was performed using a model in PROC MIXED SAS V8.0 (SAS institute, Cary, N.C.) including environment and entry as random and fixed effects, respectively. Entry-by-environment interaction was used as the error variance because the analysis for a set was based upon entry means from individual environments obtained from either the trend or lattice analysis on the basis of precision. The entry-by-environment interaction term also served as the error term used for the calculation of the LSD.

Entry means for yield from the two different sets were obtained and adjusted for sets at two levels. First, to obtain entry means over replications adjusted for sets at a given environment, and secondly, to obtain entry means across environments adjusted for sets. Adjusting entries for sets for the two levels involved a similar process, following Schutz and Cockerham (1962). Entry means from separate sets at the same environment obtained from the ANOVA utilizing either the lattice design or trend analysis were

adjusted for sets by first obtaining deviations for entry means over replications from the corresponding set mean over replications at an environment. Then, the mean of the two sets was added to these deviations.

Obtaining entry means for yield across environments adjusted for sets was performed using the LSMEANS statement in PROC MIXED in SAS V8.0. (SAS Institute, Cary, N.C.) The ANOVA for the combined analysis across environments for yield involved a model that included set, environment, and set-by-environment interaction as random effects. Entry nested within set was considered a fixed effect. The entry-by-environment nested within set interaction served as the residual variance in the model, because the analysis was based upon entry means obtained from each set and environment combination based on precision according to either the lattice or trend analysis. Entry means from the combined analysis across environments adjusted for sets for yield were obtained by subtracting the corresponding set mean across environments and then adding the mean of both sets across environments to these deviations. In addition to the combined analysis across all environments in both years, entry means for yield adjusted for set effects were obtained from a combined analysis across environments within each year.

### **Genetic parameter estimation**

Entry mean heritability estimates were obtained for metric grain yield. The target environments for grain yield included all eight environments. Commercial checks, NC300 and B104 topcrosses were excluded from the data set involved in estimation of heritability on an entry mean basis. Further, non-common RILT between the environments used to estimate heritability were dropped from the data set. The model

used to estimate heritability followed Hallauer and Miranda (1988), and included environment, set, environment-by-set interaction, rep nested within environment-by-set interaction, entry nested within set, and entry-by-environment nested within set interaction as random effects. Entry nested within set effect was treated as random here because we were interested in the estimation of the among entry variance component. The rep-by-entry nested within environment-by-set interaction served as the residual variance for the model.

The estimate for entry mean heritability for grain yield was obtained using a program written by Holland et al. (2003) in PROC MIXED in SAS V 8.0 (SAS Institute, Cary, N.C.). The delta method (Lynch and Walsch, 1998) was utilized to derive approximate standard errors for the heritability estimate. Heritability estimation involved using the harmonic mean for the total number of experimental units assigned to a given RILT, because there were two replications at all 2002 environments, three replications at three of the four 2003 environments, and two replications at the Plymouth 2003 environment (Holland et al., 2003).

### **Genotyping the RILs and linkage map construction**

Four leaf tissue samples were harvested from four different plants for each of the 143 S<sub>4:5</sub> RILs, *per se*, and for NC300 and B104, when the plants were 3-to4-wk-old. DNA was extracted according to Riede and Anderson (1996), with slight modifications (see Appendix VII).

DNA suspended in Tris-CL EDTA (TE) buffer for the four samples corresponding to the same RILs was bulked. The RILS and two parents were genotyped using 94 simple-sequence -repeat (SSR) markers in an attempt to achieve coverage at 20

cM intervals. SSR reaction procedures, conditions, and gel scoring were according to Senior et al. (1996, 1998). For further detail, please see Appendix VIII. Four percent agarose gels were set up to include one well each for NC300 and B104 reaction products as well as the 142 RILs. The RIL that had the lowest genomic DNA concentration was dropped in order to fit the two parents and the population on one and a half PCR plates in order to maximize usage of PCR machines. Digital images of gels were taken using an Eagle Eye II Still Video System (Stratagene, La Jolla, CA). Gels were scored at a given SSR marker locus by assigning a 1 or 2 corresponding to the presence of NC300 and B104 genotypes, respectively. Heterozygous lines were assigned a 3.

RILs found to be heterozygous at a given marker locus were eliminated in the construction of a linkage map and subsequent analyses involving QTL detection for that particular locus. Pearson's Chi square goodness of fit statistic (Snedecor and Cochran, 1980) was used to test if markers deviated significantly ( $P \leq 0.05$ ) from a 1:1 segregation ratio by using a macro written in PROC FREQ in SAS v8.0. Marker loci that displayed segregation distortion were not included originally in the construction of a linkage map. Mapmaker/exp 3.0 (Lincoln et al., 1993) was employed to construct a linkage map using a logarithm of the odds (LOD) threshold of 3, a 50 cM distance, and the Haldane mapping function. Marker placement was compared to the IBM2 2004 neighbors map [www.maizedb.org](http://www.maizedb.org) to identify any deviations in ordering loci. Markers that displayed significant segregation distortion prior to map construction were included in the final map only if their placement was consistent with the IBM2 2004 neighbors map.

## **QTL Detection**

Phenotypic data sets were each merged with the molecular marker data set. Phenotypic data sets included entry means for grain yield for RILT adjusted for sets obtained from analyses executed for the separate environments, across environments in years 2002 and 2003, and across all environments in both years. Composite interval mapping (CIM) was used to estimate the additive effects of genomic regions for all phenotypic datasets, and was performed using QTL-Cartographer (Wang et al., 2004). A genome-wise significance threshold ( $P = 0.05$ ) for each phenotypic data set was obtained through permutation testing following Churchill and DeGeorge (1994). Permutation testing involved 1,000 iterations for all phenotypic marker datasets. Both backward and forward selection procedures were used to perform the permutation testing and CIM, using a threshold of 0.01 for factors to enter and remain in the model. The genome was scanned every 2.0 cM. Confidence intervals were constructed for all significant genomic regions by using a 2 LOD support interval, following Mangin et al. (1994a,b). Markers identified as significantly associated with grain yield from CIM in any data set were subjected to a multi-factor ANOVA in PROC GLM in SAS V 8.0 (SAS Institute, Cary, N.C.) to test for marker-by-environment interaction. The model included environment, set, environment-by-set, rep-nested-within-environment-by-set, and marker-by-environment nested-within-set as random effects while marker-nested-within-set was considered a fixed effect.

## **Results**

### **Relative importance in accounting for spatial variation in the yield trials**

ASE values obtained from the analysis for a given set and environment, and also for the combined analysis across environments, where trend effects were fitted at individual environments, were lower than those of the corresponding lattice analysis in nine of the sixteen set-by-environment combinations for grain yield (Appendix V). Significant row and column effects involving up to the third degree polynomial were fitted at the nine set-by-environment combinations. For DTP and days to mid-silk, smaller ASE values were obtained from the trend analysis than compared to the lattice analysis for all the set-by-environment combinations (data not shown). The trend analysis also provided smaller ASE values at 11 and 6 of the 16 set-by-environment combinations for ear and plant height, respectively (data not shown). The lattice analysis produced smaller ASE values at all set-by-environment combinations for erect plants (data not shown).

### **Entry Mean Separation for Response Variables in Set 1**

Mean grain yield across environments for the RILT and commercial checks in set 1 was 6.45 and 7.22 t ha<sup>-1</sup>, respectively (Appendix III). Grain yield entry means in set 1 for the RILT ranged from 5.59 to 7.17 t ha<sup>-1</sup>. Entry means for the commercial checks across environments ranged from 6.61 to 8.81 t ha<sup>-1</sup>. The mean of the two parents in topcrosses across environments for yield was 6.55 t ha<sup>-1</sup>. NC300-T10 did not differ significantly in grain yield when compared to B104-T10 ( $P = 0.05$ ), and both yielded similarly.

Thirteen RILT in set 1 did not differ significantly ( $P = 0.05$ ) for grain yield when compared to the mean of the commercial checks. All RILT in set 1 yielded significantly lower ( $P = 0.05$ ) when compared to the highest yielding entries, P31G98 and DK697. No RILT in set 1 had grain yield significantly higher when compared to any of the commercial checks. RILT 1976-T10 and RILT 1991-T10 yielded significantly higher ( $P = 0.05$ ) than the mean of the two parents. Seven RILT yielded significantly less ( $P = 0.05$ ) when compared to the mean of the parents. Of the thirteen RILT in set 1 that did not differ significantly from the mean of the commercial checks, RILT 1976-T10 and 1991-T10 were comparable in grain yield to the commercial check, G8288. RILT 1997-T10 and RILT 1999-T10 had grain yields comparable to the commercial check, P32K61. The remaining nine yielded similarly to commercial checks LH132.51, LH195.256, P3394, and HC33.TR7322.

The thirteen RILT in set 1 that did not differ from the mean of the commercial checks were also within the range of the commercial checks for grain moisture content at harvest, ear height, plant height, days to fifty-percent pollen shed, and days to mid-silk (Table 1). Four of the thirteen RILT were significantly lower ( $P = 0.05$ ) for percent erect plants at harvest when compared to the mean of the commercial checks. 1976-T10 was significantly lower ( $P = 0.05$ ) for percent erect plants when compared to the lowest check, NK91-R9. RILT 1980-T10 had a significant longer ( $P = 0.05$ ) ASI when compared to the mean of the commercial checks. None of the thirteen RILT had a significantly longer ( $P = 0.05$ ) ASI when compared to the longest ASI of the commercial check, DK697, however.



## Entry Mean Separation for Response Variables in Set 2

Mean grain yield across environments for the RILT and commercial checks in set 2 was 6.37 and 7.21 t ha<sup>-1</sup>, respectively (Appendix IV). Grain yield entry means in set 2 for the RILT ranged from 5.41 to 7.18 t ha<sup>-1</sup>. Entry means for the commercial checks across environments ranged from 6.63 to 8.15 t ha<sup>-1</sup>. The mean of the two parents in topcrosses across environments for yield was 6.86 t ha<sup>-1</sup>. NC300-T10 did not differ significantly in grain yield when compared to B104-T10 ( $P = 0.05$ ), and both yielded similarly.

Eight RILT in set 2 did not differ significantly ( $P = 0.05$ ) for grain yield when compared to the mean of the commercial checks. ALL RILT in set 2 yielded significantly lower ( $P = 0.05$ ) when compared to the highest yielding entries, P31G98 and DK697. RILT 2070-T10 yielded significantly higher ( $P = 0.05$ ) when compared the lowest check, HC33.TR7322. No RILT in set 2 were significantly higher ( $P = 0.05$ ) for grain yield when compared to the mean of the two parents. Thirty-six RILT yielded significantly less ( $P = 0.05$ ) when compared to the mean of the parents. Of the eight RILT in set 2 that did not differ significantly from the mean of the commercial checks, RILT 2023-T10, 2070-T10, and 2074-T10 were comparable in grain yield to G8288. The remaining eight yielded similarly to commercial checks P3394, P32K61, DK743, and DK689.

The eight RILT in set 2 that did not differ from the mean of the commercial checks were also within the range of the commercial checks for grain moisture content at harvest, erect plants at harvest, ear height, plant height, days to fifty-percent pollen shed, and days to mid-silk (Table 2). RILT 2070-T10 was outside the range of the commercial

checks for ASI, but did not differ significantly ( $P = 0.05$ ) when compared to the mean of the commercial checks.

The ultimate goal is to recycle some of the RILs that did not differ significantly in topcrosses when compared to the mean of the commercial checks. The RILs were randomly derived and some RILs could be eliminated on the basis of *per se* performance from nursery data. In example, RILs 1976, 1980, and 2029 displayed poor germination and are difficult to maintain. In the Raleigh 2002 nursery, all RILs were increased by selfing 10 plants for each RIL. Non-replicated ear quality measurements were recorded for each RIL. Ear quality for RIL 2029 was poor at the Raleigh 2002 nursery (Figure 1). Ear quality for RIL 1997, 2053, and 2076 were acceptable at the Raleigh 2002 nursery (Figure 2).

### **Linkage Map**

A linkage map was constructed that included 94 markers on nine chromosomes and is displayed in Appendix I. Only three markers were obtained on chromosome six, and there was not enough information to construct a linkage map for that chromosome. The length of the map of the nine chromosomes was 1821.1 cM, with an average distance between markers of 19.37 cM. Therefore, an estimated 90% of the genome was covered if one assumes a quality map in maize is around 2000 cM (Senior et al., 1996). Nine percent of genotypic data was missing, including heterozygotes. Nine markers that deviated significantly ( $P \leq 0.01$ ) from a 1:1 segregation ratio were included in the linkage map: umc1335 (chromosome 1), bnlgl1017 (chromosome 2), umc1587 (chromosome 3), phi113 (chromosome 5), three markers located between positions 0.0 and 34.9 cM on

chromosome 7, and umc2099 and phi22 (chromosome 9). Further, marker ordering was in agreement when compared to the IBM2 2004 neighbors map.

### **Yield QTL Results**

The two years the yield trials were conducted differed drastically. The entry-by-year interaction was significant ( $P \leq 0.0001$ ) for both sets (data not shown). The 2002 year was a severe drought, while the 2003 year had above average rainfall. Therefore, analysis of putative QTL were conducted on the combined analysis across environments for both years, the combined analysis across 2002 environments, and the combined analysis across 2003 environments. In addition, analyses of putative QTL were conducted at each individual environment to further assess the consistency of putative QTL identified from the various combined analyses.

Entry mean heritability across all environments was  $0.6743 \pm 0.0417$ . Positions,  $R^2$  values, flanking markers, and effects are listed in Table 5. An allele from NC300 had an increasing effect for three putative QTL identified from the various phenotypic data sets. Phenotypic data sets where an NC300 allele was significantly associated with an increasing effect for grain yield included the combined analysis across environments in 2003, the Clayton 2002 environment, and the Plymouth 2003 environment. The putative QTL identified in the combined analysis across environments in 2003 was located on chromosome 1, and had an  $R^2$  value of 0.11 and an effect of  $0.15 \text{ t ha}^{-1}$ . The putative QTL identified in the Clayton 2002 environment was located on chromosome 2 and had an  $R^2$  value of 0.12 and an effect of  $0.26 \text{ t ha}^{-1}$ , respectively. The putative QTL identified in the Plymouth 2003 environment was located on chromosome 8 and had a corresponding  $R^2$  value of 0.12 and an effect of  $0.25 \text{ t ha}^{-1}$ , respectively.

Non-significant rises in LOD score plots occurred in other phenotypic data sets corresponding to putative QTL significantly ( $P = 0.05$ ) associated with grain yield when the NC300 allele had an increasing effect. In particular, a non-significant peak in the LOD score plot for chromosome 1 occurred in the combined analysis across all environments and the Jackson Springs 2003 environment. A non-significant rise in the LOD score plot for the chromosome 2 QTL occurred in the combined analysis across all environments. For the chromosome 8 QTL, a non-significant rise in the LOD score plot occurred at the Plymouth 2002 environment. Further, no change in rank occurred between the effects of the alleles from the two parents across the phenotypic data sets where a rise in the LOD score occurred corresponding to the three QTL significantly associated with grain yield where the NC300 allele had an increasing effect.

An allele from B104 had an increasing effect for three putative QTL identified from the various phenotypic data sets. A putative QTL located on chromosome 3 was significantly associated ( $P = 0.05$ ) with grain yield in the combined analysis across all environments. The chromosome 3 QTL had an  $R^2$  value of 0.31 and an effect of  $0.20 \text{ t ha}^{-1}$ . The QTL located on chromosome 3 was also significantly ( $P = 0.05$ ) associated with grain yield in the combined analysis across the 2002 environments, the Clayton 2002 environment, the Plymouth 2002 environment, and the Lewiston 2002 environment. Further, a non-significant rise in the LOD score plot occurred for the region located on chromosome 3 in all other phenotypic data sets except the Jackson Springs 2003 environment. No change in rank occurred between the effects of the alleles from the two parents in all phenotypic data sets where a rise in the LOD score occurred at the chromosome 3 region.

Marker bnlg1063 and umc1489 flanked the putative QTL on chromosome 3 and were separated by a distance of 33.65 cM. At the Lewiston 2002 environment, the flanking markers themselves were not significantly associated with grain yield, just the interval between the markers. Thus, care should be involved in assessing the significance of this region at this environment due to a large region in between the flanking markers for the putative QTL on chromosome3.

The second putative QTL significantly associated ( $P = 0.05$ ) with grain yield where the allele that had an increasing effect was that of B104 occurred on chromosome 7 in both the combined analysis across all environments, and the combined analysis across the 2003 environments. The QTL significantly associated with grain yield on chromosome 7 from the combined analysis across environments had an  $R^2$  value and an effect of 0.08 and 0.10 t ha<sup>-1</sup>, respectively. The putative QTL identified on chromosome 7 from the combined analysis should be viewed with caution since the position occurred in a region of segregation distortion. A non-significant rise in the LOD score plot was observed in a similar region corresponding to the QTL on chromosome 7 in other phenotypic data sets, such as the Plymouth 2002, Jackson 2003, and Lewiston 2003 environments. In addition, no change in rank between the alleles of the parents was observed for the QTL located on chromosome 7 across the phenotypic data sets where a rise in the LOD score occurred.

The third QTL significantly associated with grain yield where the B104 allele had an increasing effect was located on chromosome 9. The QTL located on chromosome 9 was only significantly associated with grain yield in the combined analysis across environments. A non-significant rise in the LOD score plot corresponding to the QTL on

chromosome 9 occurred in other phenotypic data sets, such as the combined analysis across environments in 2002 and 2003, the Clayton 2003 environment, the Jackson Springs 2003 environment, and Lewiston 2003 environment. In addition, no change in rank was observed between the alleles of the parents for the QTL located on chromosome 9 across the phenotypic data sets where a rise in the LOD score occurred.

All markers that were identified as significantly associated with grain yield from CIM exhibited significant ( $P \leq 0.01$ ) marker-by-environment interaction except for the markers flanking chromosome 3 where B104 contributed an allele with an increasing effect for grain yield (data not shown).

## Discussion

This experiment resulted in the identification of twenty-one lines that compared favorably in topcrosses when compared to the mean of the commercial checks for grain yield, percent moisture, ear height, plant height, days to mid-silk, and days to fifty-percent pollen shed. RILT 2070-T10 in set 2 significantly yielded higher ( $P = 0.05$ ) when compared to the lowest check, HC33.TR7322. Four of these lines differed from the commercial checks for percent erect plants. Two of the RILT differed from the commercial checks for ASI. Most of the twenty-one RILT are being tested in yield trials an additional year.

The two parents yielded similarly in both sets. Transgressive segregates for grain yield were observed with respect to both parents in sets 1 and 2. NC300 and B104 both possess favorable alleles for QTL associated with grain yield from the combined analyses across environments. The NC300 allele on chromosome 1 had an increasing effect of  $0.15 \text{ t ha}^{-1}$  from the combined analysis across the 2003 environments, and explained 11.0% of the phenotypic variation. The B104 allele on chromosome 3 had an increasing effect of  $0.20 \text{ t ha}^{-1}$  from the combined analysis across all environments explaining 31.0% of the phenotypic variation. B104 also had alleles associated with an increasing effect for grain yield on chromosomes 7 and 9.

Eleven of the twenty-one superior lines for grain yield possessed the B104 genotype at both loci that flanked the QTL on chromosome 3 (Appendix VI). An additional six lines possessed the B104 genotype at one of the loci that flanked the chromosome 3 yield QTL. Of the six lines, four had an unknown genotype at the other locus, whereas, two lines possessed the NC300 genotype at the other locus. RIL 1986

possessed the NC300 genotype at both flanking loci for the chromosome 3 QTL. Three lines were extremely difficult to maintain. One of these lines, RIL 2029, possessed favorable alleles at flanking markers for all grain yield QTL.

In summary, this project is part of a greater effort to develop inbred lines consisting of large amounts of exotic germplasm with the idea in mind to eventually lead to a steady flow of diverse, improved lines (Goodman et al., 2000). Several papers have suggested using QTL marker assisted selection strategies to transfer favorable segments from an exotic source to an adapted source (Tanksley and Nelson, 1996; Stuber et al., 1999). This makes great sense and has worked for highly heritable traits, such as disease resistance (Chen et al., 2000; Cregan et al., 1999; Eagles et al., 2001). Beavis (1998), Melchinger et al. (1998), and Utz et al. (2000) demonstrated that typical mapping population sizes leads to the identification of too few QTL whose effects are drastically overestimated. The problem gets worse as heritability decreases. The usage of MAS to transfer a few segments from an exotic germplasm source to an adapted source also does very little in terms of widening the germplasm base. Further, backcrossing is a conservative breeding method (Lee, 1995). Inbred lines with a large amount of tropical material in their parentage have shown potential to warrant usage as new breeding sources to U.S. commercial maize breeders (Lewis and Goodman, 2003; Tallury and Goodman, 1999; Holley and Goodman, 1988; Uhr and Goodman, 1995; Hawbaker *et al.*, 1997). Therefore, extending future gains in selection through the usage of tropical germplasm for grain yield in maize should utilize strategies that include the development of lines whose background consists of a large percentage of tropical material.



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Table 1. RILT least square entry means for response variables from the combined analysis across environments for set 1 that did not differ significantly ( $P = 0.05$ ) for grain yield when compared to the mean of the commercial checks.

Entry	Yld (bu ac <sup>-1</sup> )	Myld (t ha <sup>-1</sup> )	Moi (%)	Epl <sup>†</sup> (%)	Eht (cm)	Pht (cm)	Tass <sup>§</sup> (days)	Silk <sup>§</sup> (days)	ASI <sup>§</sup> (days)
1956-T10	108.65	6.82	16.83	93.50	95.41	256.53	70.61	70.89	0.29
1957-T10	110.22	6.91	16.14	95.60	89.43	238.44	69.01	69.89	0.89
1963-T10	111.30	6.98	15.89	94.96	91.95	254.14	69.21	69.09	-0.11
1966-T10	109.90	6.89	15.87	97.37	95.09	248.37	70.61	70.49	-0.11
1967-T10	111.02	6.97	15.93	92.56	83.43	236.53	68.61	68.89	0.29
1976-T10	114.35	7.17	16.84	89.00	90.96	263.98	70.61	71.29	0.69
1979-T10	108.84	6.83	16.81	92.83	85.71	245.08	68.61	68.89	0.29
1980-T10	111.97	7.02	16.53	96.65	86.10	260.56	69.41	70.49	1.09
1986-T10	109.85	6.89	16.79	95.09	92.44	264.57	70.81	71.49	0.69
1990-T10	108.43	6.80	15.99	95.81	86.88	246.14	68.61	68.69	0.09
1991-T10	114.19	7.16	16.72	92.73	83.95	249.63	67.81	68.09	0.29
1997-T10	111.79	7.01	16.19	95.44	92.16	259.53	68.41	68.89	0.49
1999-T10	111.79	7.01	16.44	97.80	94.05	259.22	71.41	71.69	0.29
B104.T10 <sup>‡</sup>	104.20	6.54	15.76	96.99	79.16	243.23	69.36	70.14	0.77
DK689 <sup>‡</sup>	117.04	7.34	16.62	97.15	94.99	243.21	69.36	69.81	0.44
DK697	129.66	8.13	16.42	95.63	93.22	255.62	68.61	69.09	0.49
DK743 <sup>‡</sup>	110.13	6.91	16.82	95.45	85.86	253.27	68.61	68.29	-0.31
G8288	115.61	7.25	16.31	97.34	84.58	261.57	68.01	68.49	0.49
LH132.51 <sup>‡</sup>	107.83	6.77	14.97	94.26	87.36	249.47	67.36	67.81	0.44
LH195.256 <sup>‡</sup>	106.37	6.67	16.57	97.61	94.67	251.93	70.36	70.14	-0.23
LH200.262	120.66	7.57	15.94	96.00	98.34	256.86	69.01	68.49	-0.51
NC300.T10 <sup>‡</sup>	104.74	6.57	17.18	93.20	85.28	250.54	71.03	71.81	0.77
NK91-R9 <sup>‡</sup>	116.90	7.33	18.29	93.19	105.39	289.26	74.36	71.81	-2.56
P31G98	130.45	8.18	15.60	98.52	93.35	254.09	70.21	69.29	-0.91
P32K61	111.57	7.00	16.03	97.28	78.61	249.10	68.41	68.69	0.29
P3394 <sup>‡</sup>	108.50	6.81	14.63	97.63	80.48	229.62	68.70	67.47	-1.23
HC33.TR7322	105.37	6.61	14.93	96.92	78.98	238.12	66.61	66.89	0.29
C.V.%	8.62	8.62	3.72	3.35	5.75	3.10	1.44	1.44	70.13
RILT mean	110.95	6.96	16.38	94.56	89.81	252.52	69.51	69.91	0.40
Check mean	115.01	7.22	16.09	96.42	89.65	252.68	69.13	68.86	-0.28
Parent mean	104.47	6.55	16.47	95.09	82.22	246.89	70.20	70.97	0.77
lsd1 $\alpha = 0.05$	8.86	0.56	0.60	4.77	4.87	7.62	1.97	1.98	1.57
lsd2 $\alpha = 0.05$	6.61	0.41	0.44	3.56	3.63	5.68	1.47	1.48	1.17
lsd3 $\alpha = 0.05$	8.86	0.56	0.60	4.96	4.87	7.62	1.97	1.98	1.57
lsd4 $\alpha = 0.05$	10.86	0.68	0.73	6.16	5.96	9.34	2.41	2.43	1.93

Yld = yield.

Myld = metric yield.

Moi = percent moisture.

Epl = percent erect plants.

Eht = ear height.

Pht = plant height.

Tass = days to fifty-percent pollen shed.

Silk = days to mid-silk.

ASI = anthesis silk interval.

† response variable was not measured in Plymouth 2003 environment due to a hurricane.

§ response variables measured only at the two Clayton environments.

\* T10 stands for FR615xFR697.

‡ entries present only in 2003 environments.

lsd1 = appropriate for comparing an entry mean across 2002 and 2003 environments to an entry mean across 2002 and 2003 environments.

lsd2 = appropriate for comparing an entry mean across 2002 and 2003 environments to the mean of the commercial checks.

lsd3 = appropriate for comparing an entry mean across 2002 and 2003 environments to the mean of the parents.

lsd4 = appropriate for comparing an entry mean across 2003 environments to an entry mean across 2002 and 2003 environments.

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Table 2. RILT least square entry means for response variables from the combined analysis across environments for set 2 that did not differ significantly for grain yield when compared to the mean of the commercial checks.

Entry	Yld (bu ac <sup>-1</sup> )	Myld (t ha <sup>-1</sup> )	Moi (%)	Epl <sup>†</sup> (%)	Eht (cm)	Pht (cm)	Tass <sup>§</sup> (days)	Silk <sup>§</sup> (days)	ASI <sup>§</sup> (days)
2023-T10	113.78	7.14	16.70	98.37	93.47	262.65	69.12	69.20	0.10
2029-T10	109.03	6.84	18.12	97.82	94.15	245.80	72.52	72.20	-0.30
2046-T10	109.04	6.84	16.57	96.45	88.82	252.51	70.12	71.20	1.10
2052-T10	109.21	6.85	15.92	95.27	86.09	245.18	68.52	69.00	0.50
2053-T10	110.05	6.90	17.30	92.07	89.27	254.80	69.52	70.80	1.30
2070-T10	114.44	7.18	17.65	97.16	92.36	253.85	71.12	72.60	1.50
2074-T10	113.80	7.14	15.95	96.81	82.42	245.09	69.32	70.40	1.10
2076-T10	110.53	6.93	16.16	98.57	84.58	237.14	68.92	69.60	0.70
B104.T10 <sup>‡</sup>	109.14	6.85	16.31	98.57	82.91	243.50	69.25	69.67	0.48
DK689 <sup>‡</sup>	110.80	6.95	16.93	94.94	96.30	245.77	70.91	71.33	0.48
DK697	123.07	7.72	16.64	95.77	95.38	257.65	68.92	70.00	1.10
DK743 <sup>‡</sup>	110.58	6.94	16.83	92.46	85.84	254.13	68.91	69.00	0.15
G8288	115.99	7.28	16.28	97.82	85.55	259.62	67.12	67.80	0.70
LH132.51 <sup>‡</sup>	109.94	6.90	15.28	97.03	83.45	246.21	67.91	68.67	0.82
LH195.256 <sup>‡</sup>	108.32	6.80	17.34	97.60	94.14	248.97	69.58	69.67	0.15
LH200.262	117.96	7.40	16.35	96.34	99.54	257.63	70.32	70.00	-0.30
NC300.T10 <sup>‡</sup>	109.59	6.87	17.36	96.46	84.57	251.82	69.91	70.33	0.48
NK91-R9 <sup>‡</sup>	125.88	7.90	18.69	96.24	100.25	287.20	74.58	73.67	-0.85
P31G98	129.94	8.15	15.72	98.41	94.45	256.37	69.72	70.00	0.30
P32K61	110.01	6.90	16.14	97.34	80.58	250.34	68.72	68.80	0.10
P3394 <sup>‡</sup>	110.42	6.93	14.98	95.40	80.59	232.63	68.91	69.33	0.48
HC33.TR7322	105.74	6.63	15.14	97.68	78.40	239.96	66.52	67.40	0.90
C.V.%	8.26	8.26	3.54	4.70	5.85	2.89	1.89	2.14	132.39
RILT mean	111.24	6.98	16.80	96.57	88.89	249.63	69.89	70.63	0.75
Check mean	114.89	7.21	16.36	96.42	89.54	253.04	69.34	69.64	0.33
Parent mean	109.37	6.86	16.83	97.51	83.74	247.66	69.58	70.00	0.48
lsd1 $\alpha=0.05$	8.39	0.53	0.58	3.36	4.87	7.04	2.58	2.94	1.80
lsd2 $\alpha=0.05$	6.26	0.39	0.43	2.51	3.63	5.25	1.92	2.19	1.34
lsd3 $\alpha=0.05$	8.39	0.53	0.58	3.50	4.87	7.04	2.58	2.94	1.80
lsd4 $\alpha=0.05$	10.28	0.64	0.71	4.34	5.96	8.62	3.16	3.61	2.21

Yld = yield.

Myld = metric yield.

Moi = percent moisture.

Epl = percent erect plants.

Eht = ear height.

Pht = plant height.

Tass = days to fifty-percent pollen shed.

Silk = days to mid-silk.

ASI = anthesis silk interval.

<sup>†</sup>response variable was not measured in Plymouth 2003 environment due to a hurricane.

§response variables measured only at the two Clayton environments.

\* T10 stands for FR615xFR697.

‡entries present only in 2003 environments.

lsd1 = appropriate for comparing an entry mean across 2002 and 2003 environments to an entry mean across 2002 and 2003 environments.

lsd2 = appropriate for comparing an entry mean across 2002 and 2003 environments to the mean of the commercial checks.

lsd3 = appropriate for comparing an entry mean across 2002 and 2003 environments to the mean of the parents.

lsd4 = appropriate for comparing an entry mean across 2003 environments to an entry mean across 2002 and 2003 environments.

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Table 3. Summary of grain yield QTL positions,  $R^2$ , and additive effects from the analysis at individual environments, combined analyses across environments in 2002, 2003, and all environments.

PDS	Chromosome	Left <sup>†</sup>	Right <sup>‡</sup>	Position (cM)	$R^2$	Effect <sup>§</sup> (t ha <sup>-1</sup> )
COMBALL	1	bnlg1347	bnlg2331	246.06	0.06	0.09
COMBALL	2	bnlg1175	umc1635	116.92	0.07	0.09
COMBALL	3	bnlg1063	umc1489	91.62*	0.31	-0.20
COMBALL	7	umc1409	umc1036	2.56*	0.08	-0.10
COMBALL	9	duppssr6	bnlg1401	20.94	0.03	-0.06
COMB03	1	bnlg1347	bnlg2331	246.06*	0.11	0.15
COMB03	3	bnlg1063	umc1489	81.62	0.09	-0.13
COMB03	7	umc1409	umc1036	2.56*	0.09	-0.14
COMB03	9	duppssr6	bnlg1401	18.94*	0.11	-0.15
COMB02	2	bnlg1175	umc1635	112.92	0.04	0.08
COMB02	3	bnlg1063	umc1489	99.62*	0.36	-0.27
CLY02	2	umc1555	bnlg1175	107.17*	0.12	0.26
CLY02	3	umc1489	umc1844	113.27*	0.13	-0.28
PLY02	3	bnlg1063	umc1489	95.62*	0.24	-0.27
PLY02	7	umc1409	umc1036	2.56	0.03	-0.09
PLY02	8	umc1724	umc1268	159.30	0.11	0.18
LEW02	3	bnlg1063	umc1489	99.62*	0.15	-0.24
LEW02	4	umc1051	umc1940	185.08	0.05	-0.13
JAC02	3	umc1489	umc1844	115.27	0.12	-0.21
CLY03	3	bnlg420	bnlg1063	72.63	0.06	-0.14
CLY03	9	duppssr6	bnlg1401	24.94	0.06	-0.14
PLY03	3	bnlg420	bnlg1063	70.63	0.08	-0.20
PLY03	8	umc1724	umc1268	167.30*	0.12	0.25
JAC03	1	bnlg1347	bnlg2331	246.06	0.04	0.10
JAC03	4	umc1043	umc1051	179.38*	0.13	-0.18
JAC03	7	umc1036	bnlg339	2.56	0.04	-0.11
JAC03	9	duppssr6	bnlg1401	14.94	0.04	-0.10
LEW03	3	bnlg420	bnlg1063	74.63	0.04	-0.15
LEW03	4	bnlg1137	umc1808	157.54	0.04	-0.14
LEW03	7	umc1409	umc1036	0.01	0.07	-0.19
LEW03	9	bnlg1810	duppssr6	12.01	0.05	-0.16

PDS = Phenotypic data set.

COMBALL = combined analysis across all environments.

COMB03 = Combined analysis across environments in 2003.

COMB02 = Combined analysis across environments in 2002.

<sup>†</sup>The left flanking marker for a putative QTL.

<sup>‡</sup>The right flanking marker for a putative QTL.

<sup>§</sup>Effects are expressed relative to NC300, i.e. the sign of the effect corresponds to the magnitude of the NC300 allele.

\*Indicates significance for the corresponding phenotypic data set's threshold.

Figure 1. Picture of ears harvested from RIL 2029 from the 2003 Raleigh nursery.

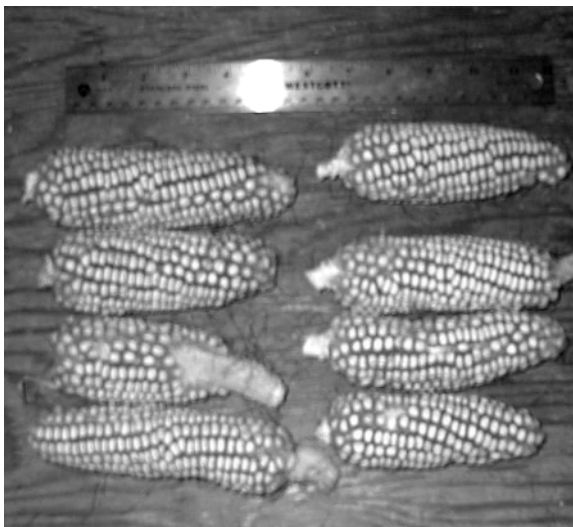


Figure 2. Ears harvested in the Raleigh 2003 nursery from RILs a) 1997, b) 2053, and c) 2076.

a) RIL 1997.



b) RIL 2053.

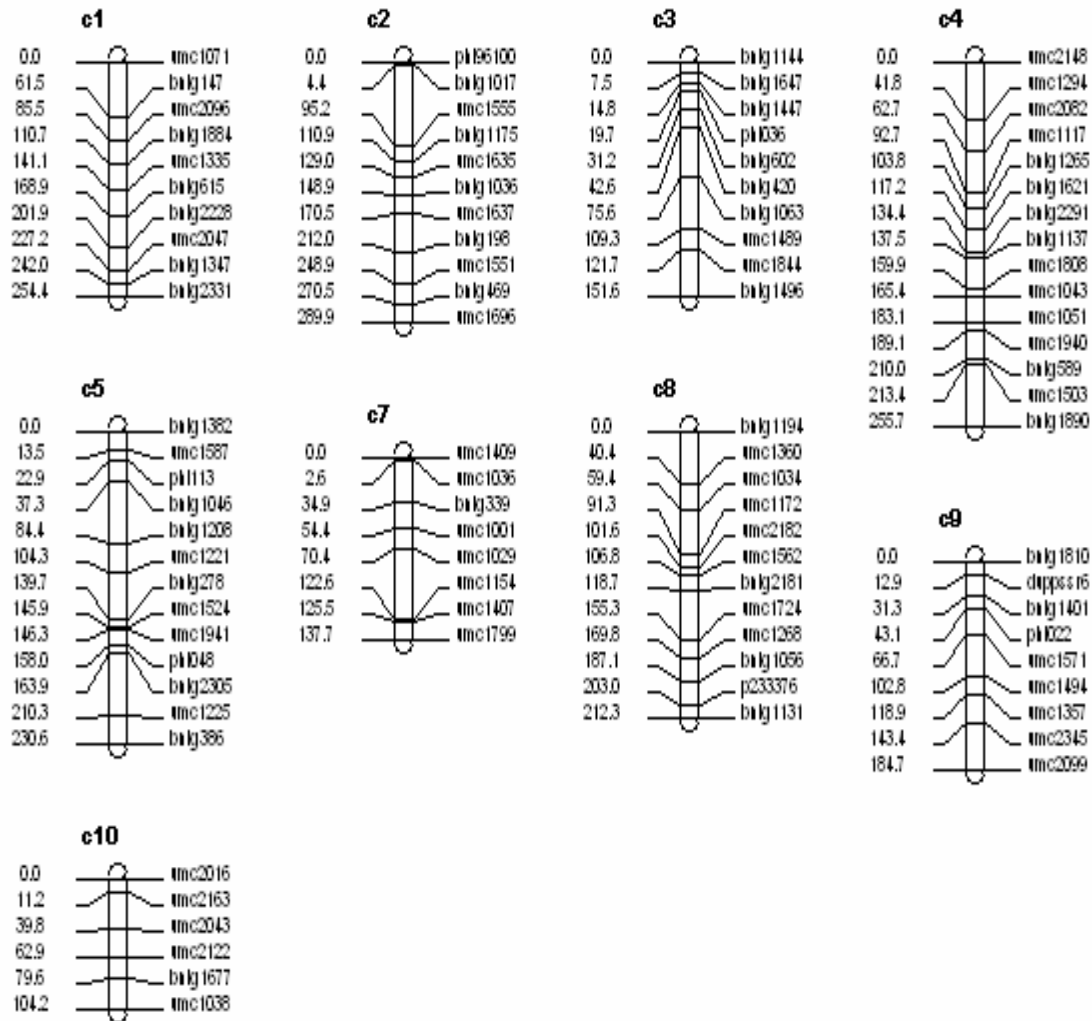


c) RIL 2076.



## **IV. Appendices**

## A I. Linkage map



## A II. Trend analysis

Rows

8	40	39	38	37	36
7	31	32	33	34	35
6	30	29	28	27	26
5	21	22	23	24	25
4	20	19	18	17	16
3	11	12	13	14	15
2	10	9	8	7	6
1	1	2	3	4	5

1      2      3      4      5      Columns

Each cell represents one plot. A column was referred to as all plots contiguous to one another along the length of the field while a row constituted all plots contiguous to one another along the width of the field. First, second, third, and fourth degree polynomials were assigned to both columns and rows.

### A III.

**RILT least square entry means for response variables from the combined analysis across environments for set 1.**

Entry	Yld (bu ac <sup>-1</sup> )	Myld (t ha <sup>-1</sup> )	Moi (%)	Epl <sup>†</sup> (%)	Eht (cm)	Pht (cm)	Tass <sup>§</sup> (days)	Silk <sup>§</sup> (days)	ASI <sup>§</sup> (days)
1952-T10*	102.28	6.42	16.92	97.34	92.39	259.68	70.21	69.89	-0.31
1953-T10	96.79	6.07	15.80	96.24	94.04	255.16	70.81	71.49	0.69
1954-T10	99.63	6.25	16.45	96.47	75.20	239.81	68.81	69.29	0.49
1955-T10	95.92	6.02	16.86	98.82	81.78	240.08	69.01	69.29	0.29
1956-T10	108.65	6.82	16.83	93.50	95.41	256.53	70.61	70.89	0.29
1957-T10	110.22	6.91	16.14	95.60	89.43	238.44	69.01	69.89	0.89
1958-T10	106.93	6.71	17.28	98.22	78.45	251.34	69.41	70.09	0.69
1959-T10	99.69	6.25	15.85	88.99	83.99	245.74	68.81	68.89	0.09
1960-T10	102.69	6.44	16.45	92.93	96.12	272.49	71.81	71.29	-0.51
1961-T10	108.18	6.79	16.36	97.26	86.86	252.94	69.81	69.89	0.09
1962-T10	100.75	6.32	15.81	97.94	85.43	244.52	68.81	68.89	0.09
1963-T10	111.30	6.98	15.89	94.96	91.95	254.14	69.21	69.09	-0.11
1964-T10	94.51	5.93	16.59	95.80	78.93	249.41	70.01	70.89	0.89
1965-T10	101.20	6.35	16.94	91.07	97.58	270.84	71.81	72.69	0.89
1966-T10	109.90	6.89	15.87	97.37	95.09	248.37	70.61	70.49	-0.11
1967-T10	111.02	6.97	15.93	92.56	83.43	236.53	68.61	68.89	0.29
1968-T10	97.10	6.09	15.62	95.39	83.59	251.67	69.21	69.89	0.69
1969-T10	106.74	6.70	15.67	89.67	84.00	243.88	69.41	69.89	0.49
1970-T10	97.18	6.10	16.47	98.68	80.39	240.11	69.01	69.49	0.49
1971-T10	103.37	6.48	16.34	99.37	79.00	241.15	68.61	69.49	0.89
1972-T10	103.67	6.50	17.21	97.18	92.73	263.57	72.21	72.29	0.09
1973-T10	100.53	6.31	16.79	94.44	76.88	247.74	68.21	69.29	1.09
1974-T10	105.82	6.64	16.68	90.13	93.04	255.80	71.61	72.29	0.69
1975-T10	101.65	6.38	16.30	91.41	84.61	256.60	69.21	69.09	-0.11
1976-T10	114.35	7.17	16.84	89.00	90.96	263.98	70.61	71.29	0.69
1977-T10 <sup>‡</sup>	89.15	5.59	16.34	98.99	83.32	250.36	68.70	69.14	0.44
1978-T10	103.97	6.52	15.78	93.70	87.10	243.45	70.61	70.29	-0.31
1979-T10	108.84	6.83	16.81	92.83	85.71	245.08	68.61	68.89	0.29
1980-T10	111.97	7.02	16.53	96.65	86.10	260.56	69.41	70.49	1.09
1981-T10	96.46	6.05	15.63	95.25	82.66	247.45	69.21	69.69	0.49
1982-T10	103.89	6.52	16.52	92.64	84.84	249.11	69.61	70.49	0.89
1983-T10	96.04	6.03	16.17	95.20	84.47	249.28	68.81	69.29	0.49
1984-T10	98.99	6.21	16.72	96.25	76.63	232.26	68.21	68.49	0.29
1985-T10	105.31	6.61	16.06	97.33	79.63	242.51	69.01	69.29	0.29
1986-T10	109.85	6.89	16.79	95.09	92.44	264.57	70.81	71.49	0.69
1987-T10	103.18	6.47	16.37	95.43	80.84	234.98	68.61	68.89	0.29
1988-T10	98.78	6.20	16.13	99.58	85.24	243.57	69.61	70.09	0.49
1989-T10	108.05	6.78	15.93	95.25	75.46	230.08	67.21	68.09	0.89
1990-T10	108.43	6.80	15.99	95.81	86.88	246.14	68.61	68.69	0.09



### A III. (Continued)

Entry	Yld (bu ac <sup>-1</sup> )	Myld (t ha <sup>-1</sup> )	Moi (%)	Epl <sup>†</sup> (%)	Eht (cm)	Pht (cm)	Tass <sup>§</sup> (days)	Silk <sup>§</sup> (days)	ASI <sup>§</sup> (days)
1991-T10	114.19	7.16	16.72	92.73	83.95	249.63	67.81	68.09	0.29
1992-T10	101.39	6.36	15.86	95.02	87.94	256.75	69.01	69.49	0.49
1993-T10	104.38	6.55	17.00	96.53	99.97	272.50	72.01	71.89	-0.11
1994-T10	105.26	6.60	16.13	94.70	81.31	244.49	68.21	68.49	0.29
1995-T10	99.33	6.23	16.53	94.92	72.09	226.70	68.41	68.49	0.09
1996-T10	101.99	6.40	16.60	98.55	87.06	257.35	69.61	69.89	0.29
1997-T10	111.79	7.01	16.19	95.44	92.16	259.53	68.41	68.89	0.49
1998-T10	106.58	6.69	16.26	98.74	80.38	233.96	69.01	68.89	-0.11
1999-T10	111.79	7.01	16.44	97.80	94.05	259.22	71.41	71.69	0.29
2000-T10	92.18	5.78	16.55	97.17	85.67	248.91	69.01	69.89	0.89
2001-T10	106.15	6.66	16.26	98.87	75.79	236.23	67.81	68.09	0.29
2002-T10	108.02	6.78	16.01	91.80	86.34	238.36	68.81	69.49	0.69
2003-T10	99.21	6.22	16.72	98.77	82.47	240.15	69.41	70.29	0.89
2004-T10	105.60	6.62	15.99	96.14	92.88	262.68	69.41	69.49	0.09
2005-T10	103.60	6.50	17.47	94.14	88.02	267.29	70.21	70.89	0.69
2006-T10	99.27	6.23	17.31	95.06	88.35	266.35	70.81	70.29	-0.51
2007-T10	103.72	6.51	16.42	98.29	87.71	257.30	72.61	72.89	0.29
2008-T10	92.95	5.83	15.81	94.13	85.07	249.65	70.21	70.29	0.09
2009-T10	95.13	5.97	15.27	93.27	77.72	231.37	68.21	68.29	0.09
2010-T10 <sup>‡</sup>	94.38	5.92	16.24	93.77	85.86	240.23	69.03	69.47	0.44
2011-T10	102.44	6.43	15.97	96.12	85.06	248.31	70.21	70.09	-0.11
2012-T10	106.15	6.66	16.32	96.24	88.12	244.04	68.41	68.49	0.09
2013-T10	99.35	6.23	16.99	96.61	91.55	265.09	70.41	70.69	0.29
2014-T10	105.29	6.61	17.24	92.87	93.91	275.45	72.01	72.49	0.49
2015-T10	97.69	6.13	15.61	96.87	87.39	253.10	71.61	71.49	-0.11
2016-T10	92.14	5.78	15.46	96.03	73.41	226.35	68.01	68.89	0.89
2017-T10	100.64	6.31	17.05	90.17	85.12	257.62	70.21	71.09	0.89
2018-T10	98.42	6.17	15.62	94.83	79.18	237.22	68.81	69.29	0.49
B104.T10 <sup>‡</sup>	104.20	6.54	15.76	96.99	79.16	243.23	69.36	70.14	0.77
DK689 <sup>‡</sup>	117.04	7.34	16.62	97.15	94.99	243.21	69.36	69.81	0.44
DK697	129.66	8.13	16.42	95.63	93.22	255.62	68.61	69.09	0.49
DK743 <sup>‡</sup>	110.13	6.91	16.82	95.45	85.86	253.27	68.61	68.29	-0.31
G8288	115.61	7.25	16.31	97.34	84.58	261.57	68.01	68.49	0.49
LH132.51 <sup>‡</sup>	107.83	6.77	14.97	94.26	87.36	249.47	67.36	67.81	0.44
LH195.256 <sup>‡</sup>	106.37	6.67	16.57	97.61	94.67	251.93	70.36	70.14	-0.23
LH200.262	120.66	7.57	15.94	96.00	98.34	256.86	69.01	68.49	-0.51
NC300.T10 <sup>‡</sup>	104.74	6.57	17.18	93.20	85.28	250.54	71.03	71.81	0.77
NK91-R9 <sup>‡</sup>	116.90	7.33	18.29	93.19	105.39	289.26	74.36	71.81	-2.56
P31G98	130.45	8.18	15.60	98.52	93.35	254.09	70.21	69.29	-0.91
P32K61	111.57	7.00	16.03	97.28	78.61	249.10	68.41	68.69	0.29
P3394 <sup>‡</sup>	108.50	6.81	14.63	97.63	80.48	229.62	68.70	67.47	-1.23

### A III. (Continued)

HC33.T7322105.37	6.61	14.93	96.92	78.98	238.12	66.61	66.89	0.29
C.V.%	8.62	8.62	3.72	3.35	5.75	3.10	1.44	70.13
RILT mean	102.87	6.45	16.35	95.31	85.69	249.64	69.57	69.94
Check mean	115.01	7.22	16.09	96.42	89.65	252.68	69.13	68.86
Parent mean	104.47	6.55	16.47	95.09	82.22	246.89	70.20	70.97
lsd1 <sub><math>\alpha=0.05</math></sub>	8.86	0.56	0.60	4.77	4.87	7.62	1.97	1.98
lsd2 <sub><math>\alpha=0.05</math></sub>	6.61	0.41	0.44	3.56	3.63	5.68	1.47	1.48
lsd3 <sub><math>\alpha=0.05</math></sub>	8.86	0.56	0.60	4.96	4.87	7.62	1.97	1.98
lsd4 <sub><math>\alpha=0.05</math></sub>	10.86	0.68	0.73	6.16	5.96	9.34	2.41	2.43
lsd5 <sub><math>\alpha=0.05</math></sub>	9.11	0.57	0.61	5.28	5.00	7.83	2.02	2.04

Yld = yield.

Myld = metric yield.

Moi = percent moisture.

Epl = percent erect plants.

Eht = ear height.

Pht = plant height.

Tass = days to fifty-percent pollen shed.

Silk = days to mid-silk.

ASI = anthesis silk interval.

†response variable was not measured in Plymouth 2003 environment due to a hurricane.

§response variables measured only at the two Clayton environments.

\* T10 stands for FR615xFR697.

‡entries present only in 2003 environments.

lsd1 = appropriate for comparing an entry mean across 2002 and 2003 environments to an entry mean across 2002 and 2003 environments.

lsd2 = appropriate for comparing an entry mean across 2002 and 2003 environments to the mean of the commercial checks.

lsd3 = appropriate for comparing an entry mean across 2002 and 2003 environments to the mean of the parents.

lsd4 = appropriate for comparing an entry mean across 2003 environments to an entry mean across 2002 and 2003 environments.

lsd5 = appropriate for comparing an entry mean across 2003 environments to an entry mean across 2003 environments.

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# A IV.

**RILT least square entry means for response variables from the combined analysis across environments for set 2.**

Entry	Yld (bu ac <sup>-1</sup> )	Myld (t ha <sup>-1</sup> )	Moi (%)	Epl <sup>†</sup> (%)	Eht (cm)	Pht (cm)	Tass <sup>§</sup> (days)	Silk <sup>§</sup> (days)	ASI <sup>§</sup> (days)
2019-T10	104.95	6.58	16.68	99.08	76.44	230.43	67.92	69.00	1.10
2020-T10	99.42	6.24	17.01	97.10	92.07	260.03	69.92	70.40	0.50
2021-T10	106.64	6.69	16.16	92.78	84.72	247.47	69.72	70.80	1.10
2022-T10	101.80	6.39	17.19	97.58	81.96	235.46	69.32	69.60	0.30
2023-T10	113.78	7.14	16.70	98.37	93.47	262.65	69.12	69.20	0.10
2024-T10	106.34	6.67	16.88	95.11	87.45	257.95	68.92	69.20	0.30
2025-T10	105.70	6.63	17.42	98.77	87.48	248.98	70.72	71.60	0.90
2026-T10	106.49	6.68	16.18	95.26	79.47	234.08	68.52	69.00	0.50
2027-T10	97.80	6.14	16.04	99.14	79.86	226.39	68.52	69.20	0.70
2028-T10	105.15	6.60	16.90	98.96	83.60	254.49	69.92	71.00	1.10
2029-T10	109.03	6.84	18.12	97.82	94.15	245.80	72.52	72.20	-0.30
2030-T10	103.72	6.51	16.50	96.38	86.48	249.22	69.52	70.20	0.70
2031-T10	99.48	6.24	17.46	97.28	85.62	254.49	68.32	69.20	0.90
2032-T10	97.43	6.11	17.33	97.84	85.85	257.58	71.52	72.00	0.50
2033-T10	103.81	6.51	16.90	98.22	87.22	244.88	69.92	70.00	0.10
2034-T10	104.28	6.54	16.61	97.22	81.74	235.32	68.32	68.60	0.30
2035-T10	99.85	6.26	16.46	96.08	83.75	243.46	68.12	69.20	1.10
2036-T10	104.29	6.54	17.17	98.45	81.30	245.19	68.52	68.80	0.30
2037-T10	108.36	6.80	16.86	96.82	75.17	237.98	69.72	70.80	1.10
2038-T10	98.74	6.19	16.47	93.81	81.03	251.03	68.52	69.80	1.30
2039-T10	99.37	6.23	15.91	95.76	83.68	253.38	68.72	69.00	0.30
2040-T10	93.62	5.87	17.44	96.91	73.26	233.19	68.12	70.40	2.30
2041-T10	96.16	6.03	17.18	97.24	82.19	254.40	68.72	69.20	0.50
2042-T10	96.91	6.08	17.46	98.55	84.40	251.32	71.72	72.00	0.30
2043-T10	100.62	6.31	16.70	97.57	81.51	246.82	69.32	70.00	0.70
2044-T10	101.34	6.36	16.36	97.00	88.76	250.30	68.92	69.60	0.70
2045-T10	94.46	5.93	16.53	95.68	81.27	246.07	68.52	69.00	0.50
2046-T10	109.04	6.84	16.57	96.45	88.82	252.51	70.12	71.20	1.10
2047-T10	99.44	6.24	17.83	95.49	77.69	238.53	69.32	70.00	0.70
2048-T10	105.01	6.59	16.80	96.80	82.16	241.75	69.52	70.80	1.30
2049-T10	107.32	6.73	16.30	89.00	90.56	256.83	69.52	69.80	0.30
2050-T10	91.77	5.76	16.84	91.87	82.58	246.47	70.12	70.60	0.50
2051-T10	102.50	6.43	16.65	95.39	93.39	258.49	69.72	70.00	0.30
2052-T10	109.21	6.85	15.92	95.27	86.09	245.18	68.52	69.00	0.50
2053-T10	110.05	6.90	17.30	92.07	89.27	254.80	69.52	70.80	1.30
2054-T10	103.69	6.50	16.94	96.26	78.21	237.07	68.52	68.80	0.30
2055-T10	104.05	6.53	17.60	94.41	90.29	263.13	70.72	71.80	1.10
2056-T10	107.79	6.76	16.60	95.31	94.36	259.15	69.72	70.00	0.30
2057-T10	99.88	6.27	16.73	92.25	87.76	260.64	71.12	72.40	1.30

**A IV.**  
**(Continued)**

Entry	Yld (bu ac <sup>-1</sup> )	Myld (t ha <sup>-1</sup> )	Moi (%)	Epl <sup>†</sup> (%)	Eht (cm)	Pht (cm)	Tass <sup>§</sup> (days)	Silk <sup>§</sup> (days)	ASI <sup>§</sup> (days)
2058-T10	96.72	6.07	16.03	98.74	80.75	239.98	68.92	70.20	1.30
2059-T10	103.97	6.52	17.88	94.00	87.93	244.99	68.92	69.20	0.30
2060-T10	97.03	6.09	15.76	95.06	81.27	240.49	69.32	69.20	-0.10
2061-T10	99.66	6.25	16.19	94.75	86.80	245.90	69.12	69.80	0.70
2062-T10	103.47	6.49	16.97	96.02	86.86	259.37	68.72	69.40	0.70
2063-T10	98.36	6.17	16.69	97.04	84.28	260.97	70.32	70.80	0.50
2064-T10	86.26	5.41	16.56	97.87	81.30	239.73	70.92	72.60	1.70
2070-T10	114.44	7.18	17.65	97.16	92.36	253.85	71.12	72.60	1.50
2071-T10	104.87	6.58	16.11	97.79	81.17	233.89	69.32	70.40	1.10
2072-T10	106.14	6.66	16.21	97.13	92.24	254.00	69.92	70.20	0.30
2073-T10	99.49	6.24	15.57	97.21	82.73	247.37	68.32	69.40	1.10
2074-T10	113.80	7.14	15.95	96.81	82.42	245.09	69.32	70.40	1.10
2075-T10	89.23	5.60	15.93	99.08	72.95	226.12	68.12	69.60	1.50
2076-T10	110.53	6.93	16.16	98.57	84.58	237.14	68.92	69.60	0.70
2077-T10	91.46	5.74	17.13	98.32	80.61	253.00	72.52	73.20	0.70
2078-T10	100.09	6.28	18.37	97.12	83.66	252.82	70.12	70.20	0.10
2079-T10	91.38	5.73	16.25	99.71	71.26	230.72	67.52	68.60	1.10
2080-T10	96.85	6.08	16.77	97.87	84.80	263.12	69.32	70.40	1.10
2081-T10	90.34	5.67	17.38	97.66	81.88	246.90	69.52	70.80	1.30
2082-T10	103.74	6.51	16.64	97.59	87.68	253.76	71.32	72.20	0.90
2083-T10 <sup>‡</sup>	98.03	6.15	16.67	98.88	76.75	236.71	69.25	70.33	1.15
2084-T10	100.61	6.31	16.13	97.79	84.09	242.75	68.92	70.40	1.50
2085-T10	98.00	6.15	16.79	98.04	76.73	240.87	68.92	69.60	0.70
2086-T10	102.87	6.45	16.30	94.20	86.34	249.94	68.92	69.60	0.70
2087-T10	105.04	6.59	15.80	92.65	86.61	243.08	68.52	69.00	0.50
2088-T10	98.72	6.19	16.56	97.28	84.75	254.42	68.72	70.00	1.30
2089-T10	101.00	6.34	16.83	97.32	84.58	250.44	71.72	72.00	0.30
2090-T10	102.80	6.45	17.04	97.64	80.22	251.20	69.12	70.60	1.50
2091-T10	103.28	6.48	17.30	98.21	80.87	252.65	68.52	68.80	0.30
2092-T10	98.43	6.18	17.06	95.40	80.43	227.90	68.32	68.80	0.50
2093-T10	94.05	5.90	16.27	98.80	85.90	242.54	69.52	69.80	0.30
2094-T10	97.38	6.11	17.03	98.71	82.16	254.31	70.52	71.20	0.70
2095-T10	103.78	6.51	16.19	97.55	76.37	241.99	69.12	69.80	0.70
2096-T10	105.69	6.63	18.25	98.72	90.64	252.20	71.52	71.60	0.10
2097-T10	95.16	5.97	16.50	98.57	80.39	244.11	68.52	69.60	1.10
2098-T10	105.59	6.62	17.14	92.38	92.55	255.94	69.92	71.00	1.10
2099-T10	100.00	6.27	16.36	97.48	82.36	242.91	68.92	69.60	0.70
B104-T10 <sup>‡</sup>	109.14	6.85	16.31	98.57	82.91	243.50	69.25	69.67	0.48
DK689 <sup>‡</sup>	110.80	6.95	16.93	94.94	96.30	245.77	70.91	71.33	0.48
DK697	123.07	7.72	16.64	95.77	95.38	257.65	68.92	70.00	1.10
DK743 <sup>‡</sup>	110.58	6.94	16.83	92.46	85.84	254.13	68.91	69.00	0.15

# **A IV.** **(Continued)**

G8288	115.99	7.28	16.28	97.82	85.55	259.62	67.12	67.80	0.70
LH132.51†	109.94	6.90	15.28	97.03	83.45	246.21	67.91	68.67	0.82
LH195.256‡	108.32	6.80	17.34	97.60	94.14	248.97	69.58	69.67	0.15
LH200.262	117.96	7.40	16.35	96.34	99.54	257.63	70.32	70.00	-0.30
NC300.T10‡	109.59	6.87	17.36	96.46	84.57	251.82	69.91	70.33	0.48
NK91-R9‡	125.88	7.90	18.69	96.24	100.25	287.20	74.58	73.67	-0.85
P31G98	129.94	8.15	15.72	98.41	94.45	256.37	69.72	70.00	0.30
P32K61	110.01	6.90	16.14	97.34	80.58	250.34	68.72	68.80	0.10
P3394‡	110.42	6.93	14.98	95.40	80.59	232.63	68.91	69.33	0.48
HC33.T7322	105.74	6.63	15.14	97.68	78.40	239.96	66.52	67.40	0.90
C.V.%	8.26	8.26	3.54	4.70	5.85	2.89	1.89	2.14	132.39
RILT mean	101.55	6.37	16.75	96.66	84.00	247.19	69.45	70.19	0.76
Check mean	114.89	7.21	16.36	96.42	89.54	253.04	69.34	69.64	0.33
Parent mean	109.37	6.86	16.83	97.51	83.74	247.66	69.58	70.00	0.48
lsd1 <sub>α=0.05</sub>	8.39	0.53	0.58	3.36	4.87	7.04	2.58	2.94	1.80
lsd2 <sub>α=0.05</sub>	6.26	0.39	0.43	2.51	3.63	5.25	1.92	2.19	1.34
lsd3 <sub>α=0.05</sub>	8.39	0.53	0.58	3.50	4.87	7.04	2.58	2.94	1.80
lsd4 <sub>α=0.05</sub>	10.28	0.64	0.71	4.34	5.96	8.62	3.16	3.61	2.21
lsd5 <sub>α=0.05</sub>	8.62	0.54	0.60	3.72	5.00	7.23	2.65	3.03	1.85

Yld = yield.

Myld = metric yield.

Moi = percent moisture.

Epl = percent erect plants.

Eht = ear height.

Pht = plant height.

Tass = days to fifty-percent pollen shed.

Silk = days to mid-silk.

ASI = anthesis silk interval.

†response variable was not measured in Plymouth 2003 environment due to a hurricane.

§response variables measured only at the two Clayton environments.

\* T10 stands for FR615xFR697.

‡entries present only in 2003 environments.

lsd1 = appropriate for comparing an entry mean across 2002 and 2003 environments to an entry mean across 2002 and 2003 environments.

lsd2 = appropriate for comparing an entry mean across 2002 and 2003 environments to the mean of the commercial checks.

lsd3 = appropriate for comparing an entry mean across 2002 and 2003 environments to the mean of the parents.

lsd4 = appropriate for comparing an entry mean across 2003 environments to an entry mean across 2002 and 2003 environments.

lsd5 = appropriate for comparing an entry mean across 2003 environments to an entry mean across 2003 environments.

# A V.

## Trend Summary for Yield

Analysis	Set	Location <sup>†</sup>	Year	MR	FV	CV	YLD Mean	ASE	DF	Factors
lattice	1	Lewiston	2002	139.18	1.61	16.19	72.85	12.92	54	R1 R2 C1
trend	1	Lewiston	2002	131.55	1.78	15.74	72.85	11.78	67	
lattice	2	Clayton	2002	104.14	2.82	8.07	126.43	10.56	64	R1 C2
trend	2	Clayton	2002	88.28	3.13	7.43	126.43	9.45	79	
lattice	2	Lewiston	2002	67.22	2.56	8.00	102.49	8.90	62	R1 R2 C1
trend	2	Lewiston	2002	74.68	2.60	8.43	102.49	8.85	76	
lattice	1	Jackson	2003	101.89	2.45	11.21	90.06	8.51	135	R1 R2 C3
trend	1	Jackson	2003	90.93	2.73	10.59	90.06	7.84	158	
lattice	1	Lewiston	2003	80.57	3.71	8.41	106.67	7.44	136	R1 C1
trend	1	Lewiston	2003	78.90	3.88	8.33	106.67	7.28	160	
lattice	2	Jackson	2003	111.67	2.20	11.98	88.22	8.93	148	R3 C1 C2
trend	2	Jackson	2003	83.85	2.77	10.38	88.22	7.60	174	
lattice	2	Lewiston	2003	99.82	4.77	10.11	98.81	8.20	150	R1 R2
trend	2	Lewiston	2003	98.00	4.94	10.02	98.81	8.11	177	
lattice	2	Plymouth	2003	130.31	3.48	6.88	166.00	9.68	138	C2
trend	2	Plymouth	2003	128.24	3.53	6.82	166.00	9.63	166	

† Only includes Location-by-year-by-set combinations where the trend analysis was more precise  
lower ASE values than compared to ASE values obtained from the corresponding lattice analysis

MR = model residual

FV = F-value for testing entry main effect

CV = coefficient of variation

LSD = least significant difference at  $\alpha = 0.05$

ASE = average standard error value for differences among all entry means

YLD Mean = Yield mean in bushels acre<sup>-1</sup>.

DF = average degrees of freedom computed from all pair-wise differences among entry means.

Effects = indicates trend effects fitted at a particular environment and set combination. C and R stand for row and column effects The number following the letter C or R indicates what degree polynomial was fitted.

## A VI.

### Genotypic data for the superior 21 lines in tropcross performance for grain yield

			YieldQTL1 <sup>†</sup> Chrom 1	YieldQTL2 Chrom 2	YieldQTL3 Chrom 3	YieldQTL4 Chrom 7	YieldQTL5 Chrom 9	GLSQTL1 Chrom 4	SALGLSQTL2 Chrom 8							
Position (cM)			42.0	254.4	110.9	129.0	75.6	109.3	0.0	2.6	12.9	31.3	103.8	117.2	40.4	59.4
RIL	MYLD	GLS	b1347 <sup>‡</sup>	b2331	b1175	u1635	b1063	u1489	u1409	u1036	dupsr6	b1401	b1265	b1621	u1360	u1034
1956	6.82	7.17	- <sup>\$</sup>	-	+	+	-	-	-	-	-	-	+	+	-	+
1957	6.91	7.07	+	+	+	-	-	-	-	-	-	-	-	-	+	+
1963	6.98	7.35	+	+	+	+	+	-	+	m	-	+	-	-	-	h
1966	6.89	7.19	-	h	-	+	-	-	-	-	-	-	+	h	-	+
1967	6.97	6.65	-	-	-	+	-	-	-	-	+	+	-	-	-	-
1976	7.17	7.43	m	-	+	+	-	+	+	-	-	-	+	+	-	-
1979	6.83	6.14	+	+	+	m	m	-	+	+	+	+	-	-	+	+
1980	7.02	7.46	m	+	+	+	+	m	-	-	-	+	m	m	+	+
1986	6.89	7.63	+	+	+	+	+	+	+	+	-	-	+	+	-	+
1990	6.80	7.24	-	+	+	+	-	-	-	-	-	-	+	+	-	-
1991	7.16	7.83	-	-	-	+	-	-	-	-	+	+	+	+	+	+
1997	7.01	7.13	+	h	-	-	+	-	-	-	+	+	-	-	-	+
1999	7.01	7.27	+	+	+	m	m	-	+	+	-	-	-	+	+	+
2023	7.14	7.37	+	+	-	m	-	-	+	+	+	+	-	-	+	+
2029	6.84	6.89	+	+	-	m	-	-	-	-	-	+	+	+	-	h
2046	6.84	7.58	m	+	m	-	m	-	-	h	+	+	-	-	+	+
2052	6.85	7.21	+	+	-	-	-	-	-	-	+	+	+	-	-	-
2053	6.90	7.29	+	-	+	+	-	-	+	+	+	+	-	-	-	-
2070	7.18	7.55	-	-	+	+	-	-	+	+	-	-	+	+	-	+
2074	7.14	6.50	+	+	+	+	-	+	-	-	m	-	h	-	+	m
2076	6.93	7.00	+	+	-	m	-	+	+	+	+	-	-	-	+	+
Position	(cM)		42.0	254.4	110.9	129.0	75.6	109.3	0.0	2.6	12.9	31.3	103.8	117.2	40.4	59.4

## A VI. (continued)

		LASGLSQT11		GLS*	DTPQT11	DTPQT2		DTPQT3			
		Chrom 1		Inter	Chrom 4	Chrom 8		Chrom 1			
Position (cM)		0.0	61.5		165.4	183.1	106.8	118.7	201.9	227.2	
RIL	MYLD	GLS	u1071	b147	*	u1043	u1051	u1562	b2181	b2228	u2047
1956	6.82	7.17	+	-	+	+	+	+	-	+	-
1957	6.91	7.07	-	-	+	-	-	-	+	+	+
1963	6.98	7.35	m	+	+	-	-	+	-	+	+
1966	6.89	7.19	+	+	+	-	-	+	+	-	+
1967	6.97	6.65	-	m	-	-	-	m	-	-	-
1976	7.17	7.43	m	+	+	+	+	+	+	-	-
1979	6.83	6.14	-	m	+	+	-	-	-	+	-
1980	7.02	7.46	-	+	+	m	+	-	-	+	+
1986	6.89	7.63	-	+	+	+	+	-	-	+	+
1990	6.80	7.24	-	m	+	+	-	-	-	-	-
1991	7.16	7.83	-	m	+	+	-	+	-	-	-
1997	7.01	7.13	-	m	+	-	-	+	+	-	+
1999	7.01	7.27	+	+	+	-	-	+	+	+	+
2023	7.14	7.37	+	+	+	-	-	+	m	-	+
2029	6.84	6.89	-	+	+	+	+	+	-	-	+
2046	6.84	7.58	+	+	m	+	+	m	-	-	-
2052	6.85	7.21	+	+	-	-	-	-	-	-	+
2053	6.90	7.29	-	+	+	-	+	-	-	+	+
2070	7.18	7.55	-	+	+	+	+	-	-	+	+
2074	7.14	6.50	+	+	+	m	-	+	+	+	+
Position	(cM)	0.0	61.5			165.4	183.1	106.8	118.7	201.9	227.2

MYLD=Entry mean metric yield in tons ha<sup>-1</sup> over all environments not adjusted for set effects.

GLS=Entry mean Gray Leaf Spot rating from a 1 to nine scale (1=susceptible and 9=resistant) not adjusted for set effects.

†Genotype data for QTL associated with grain yield, GLS resistance, and DTP given for the superior 21 lines for grain yield. Grain yield QTL have YLD as part of their nomenclature, whereas GLS and DTP QTL have GLS and DTP in their nomenclature, respectively.

‡Genotypic data is included for flanking markers for each QTL.

§ - indicates B104's genotype, whereas, + indicates NC300's genotype, m indicates missing data, and an h indicates heterozygous.

\*GLS Inter = GLS interaction. A + was assigned if a line possessed NC300 genotype at either the b1175 or u2182 loci, or at both loci. Otherwise, a - was assigned if a line possessed the b104 genotype at both loci.



## A VII.

### Monocot DNA Isolation

#### Micro-Sample Size

1. Place fresh tissue in collection tubes and transport to lab on ice. You will grind the samples with a Red Devil paint shaker located in 2108 Williams Hall.
2. Add 600 µls of extraction buffer to each tube and incubate at 65<sup>0</sup>C for 30-40 mins. Invert samples every 10 mins.
3. Spin tubes for 10 minutes and transfer supernatant into new collection tubes with caps.
4. Add 600 µls of chloroform:isoamy alcohol (24:1) to sample tubes and invert 3-4 times to mix. **Make sure caps are securely in place before this step is attempted.**  
 \*\*At this stage Rnase A (10mg/ml) is added to each sample and incubate for 30 minutes.
5. Centrifuge for 10 mins. at 3000 rpm in the Sorval centrifuge in 2105 Williams Hall. Aspirate the top layer and place in clean round bottom 96-well storage plate. Be sure not to disturb the interface. It **IS NOT** necessary to aspirate all of the upper phase; this will only increase your 230-nm readings not your final DNA concentration. **This step may be performed twice to decrease 320 nm readings but a loss in yield will be seen. It may be necessary to perform this step for ABI data collection.**
6. Fill the samples with cold 95% ethanol and mix to precipitate the DNA. Place in a -20<sup>0</sup>C freezer for approximately 1 hour. If longer, then samples precipitate crap.
7. Centrifuge samples for approximately 5 minutes and gently pour off the 95% ethanol and add 300 µls of 70% ethanol. Invert 3-4 times, centrifuge and pour off. If you leave the 70% ethanol for 1hr. to overnight samples will clean up more. At this point you will need to dry the samples. This can be done by placing the sample tubes in an oven at 65 degrees centigrade (keep checking the samples and be sure not “cook” your sample. 10-15 mins. is enough.).
8. Add 200 µls of 1X TE to each sample shake in incubator/shaker in 2114 Williams to dissolve and place in a 4<sup>0</sup>C refrigerator to store.
9. Read samples on spectrophotometer and dilute.

#### Extraction Buffer

Reagents	100 mls	200 mls	500 mls	1 liter
5M NaCl	10 mls	20 mls	50 mls	100 mls
1 M Tris-HCl (pH 8.0)	10 mls	20 mls	50 mls	100 mls
0.5 M EDTA (pH 8.0)	5 mls	10 mls	25 mls	50 mls
20% SDS	4.2 mls	8.2 mls	21 mls	42 mls

**A VII.**  
**(Continued)**

Fill to final volume with distilled water. **Note:**Add 0.38 gms. of Sodium Bisulfite/100 mls of buffer before use and adjust final pH to 7.8-8.0 with 10N NaOH. Pre-heat buffer to 65<sup>0</sup> C.

**1X TE**

Reagents

	<u>1liter</u>
1 M Tris	10 mls
0.50 M EDTA (pH 8.0)	2 mls
distilled water	988 mls

## AVIII.

### Simple Sequence Repeats in Maize- a simplified approach

**Before beginning, carefully quantify all primers and template DNA.**

#### Primer Dilution

There are numerous manufacturers of oligos in the United States and the concentrations are somewhat different. If you follow the steps below there should be no problem obtaining results in the lab.

#### Research Genetics

These primers are shipped to us at a concentration of 20  $\mu\text{M}$ . To make up a working solution (0.5  $\mu\text{l}$ /reaction) dilute selected primer 1:4 with sterile distilled water (1  $\mu\text{l}$  primer + 3  $\mu\text{l}$  sterile distilled water). Remember to use 0.5  $\mu\text{l}$ /reaction of each primer (forward and reverse).

#### Gibco or Genosys or MWG

These primers are shipped dry (powder) and diluted in 1X TE to a concentration of 1  $\mu\text{g}/\mu\text{l}$ . This is the concentration you find the primers in the freezer. They must be diluted to a working concentration of approximately 50  $\text{ng}/\mu\text{l}$ . Remember to use 0.5  $\mu\text{l}$ /reaction (25 $\text{ng}$ ) of each primer (forward and reverse). This can be accomplished with a 1:20 dilution (1  $\mu\text{l}$  primer + 19  $\mu\text{l}$  sterile distilled water). I found a 1:25 dilution to work quite well in my reactions.

The basic reaction mix (**for 96 well plate machines**)

Final concentration reaction	Stock solutions	Per 15 $\mu\text{l}$
.2 $\mu\text{M}$ forward primer	5 $\mu\text{M}$	.5 $\mu\text{l}$
.2 $\mu\text{M}$ reverse primer	5 $\mu\text{M}$	.5 $\mu\text{l}$
1 unit Taq polymerase	n/a	0.2 $\mu\text{l}$
100 $\mu\text{M}$ each dNTP	3.0 mM	.5 $\mu\text{l}$
1X reaction buffer	10X	1.5 $\mu\text{l}$
Non-acetylated BSA	10 $\text{mg}/\text{ml}$	1.5 $\mu\text{l}$
50 $\text{ng}$ template DNA	10 $\text{ng}/\mu\text{l}$	5.0 $\mu\text{l}$
1.0mM $\text{MgCl}_2$	15mM	1.0 $\mu\text{l}$
dd $\text{H}_2\text{O}$	to 15 $\mu\text{l}$	4.3 $\mu\text{l}$

**\*\*If  $\text{MgCl}_2$  is not added to the reaction, just replace it with an additional 1  $\mu\text{l}$  of sterile distilled water.**

Add DNA template to plates. Make master mix consisting of all other components. Add 10  $\mu\text{l}$  of master mix to each reaction well. Spin down with a table top centrifuge and

## **A VIII.**

### **(Continued)**

place in a thermocycler. Seal with appropriate lid and set for the following program:  
**SSR3**

**Below is a list of cycles for information only. At no time are you allowed to change these settings. If you wish to deviate from the listed protocol please see David and arrangements will be made for you to enter another program to fit your needs.**  
**Thank you.**

5 min @ 95° C

Then begin a "touch-down" protocol as follows:

1 min @ 94° C

1 min @ 65° C

2 min @ 72° C

For two cycles, then:

1 min @ 94° C

1 min @ 64° C

2 min @ 72° C

For two cycles, then:

continue dropping the annealing temperature by 1 degree C, each time repeating for two cycles, until an annealing temperature of 55°C is reached. Repeat this last set of conditions for 20 cycles to be followed by a soak cycle of 4° C. When reactions are complete, add 10 ul of Agarose bead loading dye. Load 24 ul of each reaction (avoid drawing up the oil) onto a 4% Metaphor agarose gel (1 mm combs work best), made with 1X TBE, to which you have added 10 ul of 10 mg/ml Ethidium Bromide solution. We use at least (2) 50bp ladders/row as markers. Run the gel for 4 hours at 100 volts (350 ml gel) or 150 volts (500 ml) in 1X TBE running buffer.

### **4% SFR Agarose Gel.**

#### **350 ml gel:**

350 mls 1X TBE running buffer

14 gms (Metaphor agarose) This is a high resolution agarose

Add 350 mls 1X TBE plus a large stir bar (3 inches) to a 2-liter Erlenmeyer flask.

Rapidly stir (setting 5 on the stir plate) the buffer while s-l-o-w-l-y adding 14 grams of Metaphor. Cover the top with saran wrap, poke in a few holes and microwave on high

## **A VIII.**

### **(Continued)**

for 4 minutes. Stir until cooled to 55-60° C, add the Ethidium bromide and pour with 1X4 mm combs out. Place combs in tray, let cool until polymerized and place in refrigerator for at least 30 minutes prior to loading.

#### **500 gms gel**

500 mls 1X TBE running buffer  
20 gms (Metaphor agarose)

See above instructions for proper mixing guidelines.

These gels can be reused numerous times depending on the level of polymorphism. But the minimum should be 3 times.

#### **To make the stock and working solutions of the dNTPs:**

Start with a 100mM solution of each dNTP (most manufacturers). Take 125 ul of each solution and add it to 500 ul of dH<sub>2</sub>O. This is your **Stock** solution. To make the **Working** solution (the one you will add to the PCR reactions), take 100 ul of the Stock solution and add it to 300 ul dH<sub>2</sub>O (1:4).

\*\*\*We use Gibco Taq polymerase and non-acetylated (now called purified BSA from New England Biolabs).