IDENTIFICATION AND MAPPING OF QUANTITATIVE TRAIT LOCI CONFERRING DISEASE AND INSECT RESISTANCES IN MAIZE

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ABSTRACT

Molecular markers were used to identify quantitative trait loci (QTLs) conferring resistance to three diseases and three insect pests in 110 maize recombinant inbred lines (RILs). The markers included 116 restriction fragment length polymorphisms (RFLPs) and four simple sequence repeats (SSRs). The 110 RILs were derived from a cross between Hi34 (an Antigua 2D conversion) and TZi17 (a Nigerian inbred) by single seed descent (SSD) procedure. Significant differences among the parents and significant departures from normality with regard to these diseases and pests of the RIL populations served as the basis for further analysis and QTL mapping. The RIL data were analyzed to determine the chromosomal locations of QTLs by the use of QTL Cartographer version 1.12 and single factor analysis of variance (SAS GLM).

The three corn diseases evaluated include maize streak virus (MSV), head smut (*Sphacelotheca reiliana* (Kühn) Clint), and common rust (*Puccinia sorghi* Schw.). The three insect pests studied were the corn leaf aphid (*Rhopalosiphum maidis* (Fitch)), fall armyworm (*Spodoptera frugiperda* (J. E. Smith)), and sugarcane borer (*Diatraea saccharalis* (Fabricius)). Insect and disease nurseries of the RILs were planted or had been previously planted at International Institute of Tropical Agriculture (IITA) in Nigeria, International Corn and Wheat Improvement Center (CIMMYT) in Mexico, Pioneer Co. in South Africa, and Waimanalo, Hawaii from 1992 to1998.

Composite interval mapping located a major QTL conferring resistance to MSV, previous named *msv1*, and a major QTL conferring resistance to *Sphacelotheca reiliana*

(Kühn) Clint, designed as *spr1*, on the short arm of chromosome 1 between *asg30* and *umc167*. The two genes were about 12 cM apart and both originated from Nigerian parent TZi17. Each explained 29.6% and 10.6% of the phenotypic variations, respectively.

Two QTLs, designated as *qrp1* and *qrp2* with general resistance to *Puccinia sorghi* Schw., were mapped to chromosomes 6 and 9, respectively.

A major gene conferring resistance to corn leaf aphid, designated as *aph2*, was mapped on short arm of chromosome 2 with about 14.3% phenotypic variation explanation. Seven and three QTLs were identified for resistance to fall armyworm and sugarcane borer, respectively.

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CHAPTER ONE

GENERAL INTRODUCTION

Quantitative genetics deals with the inheritance of metrical or quantitative traits that are often influenced by many genes and environmental effects. Until they can be precisely identified as genes, quantitative traits are mapped to chromosomal regions and referred to as quantitative trait loci or QTLs. QTLs that associated with economically important traits such as plant insect and disease resistance have been described statistically in the past by progenies such as diallel cross analysis and generation mean analyses. It has become feasible through molecular genetics to define the location of individual QTL on chromosome and often to describe their specific effects.

Breeding for insect resistance in corn is very important due to concern about pesticides and the environment. Three major components of pest resistance are antibiosis, preference and tolerance. The mapping of QTLs for resistance to pests can aid traditional breeding through the incorporation of resistance genes into elite corn hybrids.

Diseases are major limiting factors to crop yield worldwide. The use of resistant cultivars is the most economical and effective way of controlling their epiphytotics. Two major types of disease resistance are exploited to reduce disease. These are vertical (often monogenic) and horizontal (usually polygenic) resistance. Vertical resistance is racially specific, simply inherited, and in theory is easy to identify and to manipulate. It is also

prone to being negated due to evolution of pathogen races. Horizontal resistance is not racially specific and tends to be more stable and enduring than vertical resistance. Resistance is considered durable when it remains unaffected by evolution of the pathogen, despite widespread cultivation in an environment favoring this disease. Durable resistance is variously controlled by single gene or multiple genes depending on the different pathosystems, and the resistance may be either complete or partial.

The genetic basis of general resistance to many diseases is still not well understood. Although considerable progress has been made, attempts to transfer general disease resistance QTLs among plants have not been widely successful due to the complexity of the trait and limitations of the traditional research methodologies used.

The basic principle in identifying a QTL is by its linkage with a genetic marker. One exciting development in quantitative genetic analysis is the use of molecular techniques to uncover an essentially unlimited number of polymorphic molecular markers. The first molecular markers used were isozymes, protein variants detected by difference in migration on starch or polyacrylamide gels. Isozymes have been extensively used in population genetics since the 1960s, but they are difficult to use for high-resolution mapping of QTLs. New sources of high quality polymorphic markers are based on the DNA level and have developed rapidly since mid 1980s. The most common of these for QTL studies are restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs), and simple sequence repeats (SSRs).

Resolution of a quantitative trait into major QTLs can often explain the largest proportion of phenotypic variation. Detection and mapping of major QTLs should become of great value to breeders through the introgression of such QTLs. This can facilitate the traditional breeding program and make more efficient use of exotic plant germplasm in crop improvement.

The objectives of this research on maize were: (1) To detect major QTLs conferring disease and insect resistance segregating in the recombinant inbred lines (RILs); (2) To map QTLs conferring disease and insect resistance using polymorphic molecular markers; (3) To characterize the identified QTLs in the response to disease and insect stress. It is intended that this research be useful both in elucidating the inheritance mechanism of resistance and in future maize improvement.

CHAPTER TWO

LITERATURE REVIEW

2.1. Quantitative Trait Loci (QTLs)

Quantitative trait loci (QTLs) are chromosomal regions containing genes that affect quantitative or metrical traits (Falconer and Mackay, 1996). Major QTLs refer to QTLs with relatively large phenotypic effect (10-40%). The detection and mapping of major QTLs are important both in breeding application and genetic analysis. Detection of major QTLs is the first step, and often essential in initiating a molecular mapping program (Falconer and Mackay, 1996).

2.1.1. Major QTLs and Their Detection

The basic historic model of quantitative genetics is that the inherited differences between individuals are due to many unlinked genes. Each of these genes have small and equal effect on the phenotype, and these effects are additive. The modern view recognizes that measurements made on any quantitative trait represents the combination of all segregating QTLs and an environmental deviation that may include genotype-environment interaction (Falconer and Mackay, 1996).

There are many problems with the historic assumption that all QTLs have an equal effect on phenotype. Robertson (1989) suggested that the distribution of QTLs effect is highly leptokurtic, with a few QTLs having large effect (major QTLs) and most others

having small effects (minor QTLs). Evidence from *Drosophila*, mice, and many plant and animal species support this hypothesis. Brewbaker (1995) suggested that many quantitative traits are monogenic and that multiple allelism and linkage constitute major amendments to the historic model.

Major QTLs responsible for economically important characters are frequent in the plant kingdom (Arus and Moreno-Gonzalez, 1993). Disease resistance, male sterility, self-incompatibility and other traits related to the shape, color and architecture of plant are of mono or oligogenic nature (Arus and Moreno-Gonzalez, 1993). Clearly major QTLs should be considered in models of quantitative genetic analysis, and finding and incorporating these major QTLs can be of significance in plant breeding programs.

The most powerful tests for the presence of major QTLs are those based on information from linked markers. With the development of molecular mapping techniques, mapping major QTLs is considerably easier when major QTLs exist. Mapping programs can also suggest the proportional phenotypic effect of major QTLs on the quantitative trait.

Phenotypic information is the initial step in initiating a molecular mapping program. Without linked marker information, there are problems in detecting major QTLs by quantitative genetic analysis. It is difficult to dissociate major QTLs from the other QTLs influencing the same quantitative trait. The effects of segregating major QTLs can also be obscured if environment variation is large relative to the effects of any individual QTL or if major QTLs are at low frequency.

Detection of major QTLs is facilitated considerably with designed experimental populations such as F_2, F_3 populations and recombinant inbred lines (RILs). A quantitative trait will usually follow a single normal distribution in the absence of major QTLs. When a major QTL is segregating, the phenotypic distribution can show departure from normality such as bimodality, skewness and/or kurtosis. Departure from normality can be an indication of the presence of major QTLs, and such a mixture model forms the basis for a variety of tests for identifying major QTLs (Brewbaker, 1995).

2.1.2. Detecting Major QTLs in the RILs

Recombinant inbred lines (RILs) are produced from the F_2 progeny of two progenitor inbred lines. After six or more generations of single seed descent (SSD) by selfing (or sibling), the RILs become homozygous for short linkage blocks of progenitor alleles. RILs have long been used in mouse genetics for linkage determination (Bailey, 1981). In plants, RILs have also been constructed and used for estimations of the component of variances (Jinks, 1981), in plant breeding (Brim, 1966) and for QTL mapping (Burr, 1988).

Ten sets of maize RILs from 12 parents of tropical and temperate origin have been developed at the University of Hawaii at Manoa (Moon, 1995; Moon *et al.*, 1999). These RILs were self-pollinated using the single seed descent (SSD) method. They were studied to identify QTLs conferring disease resistance, insect and stress tolerance, and a host of agronomic traits.

Deviation from normal distribution is the initial basis for identification of the major QTLs (Le Roy and Elsen, 1992). Brewbaker (1995) developed a method employing a normal distribution curve to predict the major QTLs. This method is based on the assumption that the distribution of RILs is a mixture of the two parents and any recombinant genotype (and thus a mixture model). The parental means and variances are used to predict the distribution of segregating progeny based on monogenic, digenic and polygenic models. The expected distribution is compared with the experimental distribution, and Chi-square and least-square estimates are used to test the presence of major QTLs. Both quantitative genetic analysis and QTL mapping confirmed this method for identifying major QTLs governing disease resistance and several agronomic traits of maize (Moon, 1995; Ming, 1995).

2.2. Molecular Markers

QTLs are mapped by the use of association between characters and marker alleles (Patterson *et al.*, 1988). The first marker loci available were those that have an obvious effect on plant morphology. Sax (1923) crossed inbred bean lines differing in seed pigment and weight, with the pigmented parents having heavier seeds than that of non-pigmented parents. These crosses demonstrated that seed pigment is linked to factors that act in an additive fashion on seed weight. This hypothesis was confirmed recently using molecular mapping method (Johnson *et al.*, 1996). Brewbaker (1974) suggested the linkage of a maize mosaic virus resistant gene to morphological markers on chromosome 3 (*lg*2 and

*na*1) based on linkage evident in backcross conversion, and this result was confirmed through molecular mapping program (Ming, 1995). The problem in mapping QTLs by phenotypic markers is the limited availability of the number of markers (Staub *et al.*, 1996). With the advancement of molecular genetic techniques, molecular markers are now widely available and these markers have been used in QTL mapping, including protein level markers (isozymes) and DNA level markers (Tanksley, 1995). DNA markers include RFLPs derived from DNA digested using restriction enzymes, and PCR-based DNA segments replicated by polymerase chain reactions (PCR).

2.2.1. Isozymes

The first molecular markers used in genetic studies were polymorphic gene products, the isozymes (Marker and Moller, 1959). The paucity of isozyme loci and the fact that they are subject to post-translational modifications often restrict their utility (Staub *et al.*, 1982).

2.2.2. RFLPs

The development of molecular marker techniques has provided a method for mapping allelic variation without identifying the gene products. This method makes use of the fact that single base changes in the recognition sequence of restriction enzymes can alter the pattern of cuts made in DNA. This gives rise to a detectable variation in DNA fragment length that is inherited in a Mendelian co-dominant fashion. These allelic variants

(polymorphisms) are called restriction fragment length polymorphisms or RFLPs (Helentjaris *et al.*, 1986).

The use of RFLPs in QTL mapping requires a collection of cloned DNA segments (DNA probes) that recognize variations in enzyme cutting sites, and the mapping of these sequences to specific chromosomes. DNA probes that include highly repetitive DNA sequences are not suitable as they hybridize with a large number of DNA fragments. Therefore, unique DNA sequences are preferred as probes in detecting RFLPs. Two methods are used in obtaining unique sequence probe, cDNA clones and genomic clones (Tanksley, 1993).

Genotyping protocol of RFLP analysis is briefly described as follows. Genomic DNA is first collected from tissue samples, digested using a variety of restriction enzymes, then the cut (digested) DNA is separated by electrophores on agarose gel. Following electrophoresis, the DNA is denatured and blotted onto a nylon membrane. Probes are labeled by random priming. Hybridization is conducted in an oven and then the membrane undergoes a series of stringency washes and exposure to x-ray film (Hoisington *et al.*, 1994)

2.2.3. PCR Based DNA Markers

The Polymerase Chain Reaction (PCR) has been used to develop several DNA marker systems. The principle of PCR depends on the observation that DNA replication requires a short primer sequence. The PCR technique involves three steps: (1)

denaturation of double-stranded DNA by heating, (2) annealing the extension primers to a site flanking the region to be amplified, and (3) primer extension, in which strands complementary to the region between the flanking primers are synthesized.

Three types of DNA markers have been developed using this striking new technology. The first type includes markers that are amplified using single primers in PCR, such as Random Amplified Polymorphism DNAs or RAPDs (Williams *et al.*, 1990). The second type includes markers that are selectively amplified with two primers in PCR, such as Amplified Fragment Length polymorphisms or AFLP (Zabeau and Vos, 1993). The third type uses flanking primers of specific segments in PCR, such as simple sequence repeat, SSRs (Rafalski and Tingey, 1993).

An RFLP procedure requires a tedious process of the cloning of fragments, southern blotting and autoradiographing of gels (Zabeau and Vos, 1993). In contract, PCR based DNA markers are easily identified by staining electrophoresis gels containing fragments synthesized in a few hours using the automated technology of PCR. A further advantage is that PCR based DNA markers require a very small quantity of target DNA and thus tolerate crude extraction. PCR based DNA markers are being developed very rapidly in molecular mapping programs.

2.2.3.1. Random Amplified Polymorphism DNAs (RAPDs)

A RAPDs procedure usually uses short synthetic deoxyribonucleotides of random sequence as primers for PCR (Williams *et al.*, 1990). The PCR products are produced

from random regions of the genome. These primers identify polymorphisms in the presence or absence of specific nucleotide sequence information. RAPDs analysis usually includes three steps: genomic DNA isolation; PCR amplification; and analysis of the amplification products by agarose gel electrophoresis.

A major limitation to the use of RAPDs is that they are dominant markers, so marker genotype is ambiguous (e.g., MM and Mm cannot be distinguished) in QTL mapping. This is especially apparent when using F_2 and backcross populations.

2.2.3.2. Amplified Fragment Length Polymorphisms (AFLPs)

Production of amplified fragment length polymorphisms (AFLPs) is based on selective restriction enzyme digested fragments. Multiple bands can be generated through the amplification reaction that contains DNA markers of random origin. Heterozygous and homozygous genotype can be differentiated by the quantitative analysis of the intensity of the amplified bands. AFLPs are less used in mapping programs due to the high cost of this privately licensed marker system (Zabeau and Vos, 1993).

2.2.3.3. Simple Sequence Repeats (SSRs)

Simple Sequence Repeats (SSRs) are a subset of the tandemly repeated DNA family, represented by extremely short nucleotide sequence repeats that are abundantly present in eukaryotic genomes. The discovery of SSRs, combined with the ability to observe repeat length variation by means of the PCR technique using conserved flanking

regions, have made SSRs a useful DNA markers (Rafalski and Tingey, 1993).

The genotyping protocol of SSR analysis is quite similar with that of RAPDs. Instead of using random sequence as primers, SSR analysis uses specially designed primers for PCR amplification. A few reports have demonstrated the feasibility of using SSRs in both germplasm analysis and genetic mapping (Zietkiewicz et al., 1994). The positive features include the random distribution throughout the genome, the large allelic variation, the co-dominance and the ease of use. These make SSRs the preferred markers for future mapping of genomes and QTLs mapping.

2.3. Experiment Design for QTLs mapping

The idea in using polymorphic molecular markers for mapping QTLs is straightforward. If marker and QTL alleles are linked, differences in the trait distribution across the marker genotypes can provide information on the linkage. The following is a review of several experimental designs that generate disequilibrium between markers and QTL alleles in the inbred line crosses, and use such disequilibrium in identifying QTL-marker association.

2.3.1. Mapping Populations.

Two key components required for QTL mapping using linked markers are that individuals (1) show disequilibrium between QTLs and linked markers and (2) are informative (doubled heterozygous MQ/mq is preferred, here M/m stand for markers, Q/q

stand for QTLs). Both of these can be satisfied using F_1 parents from crosses between two inbred lines fixed for alternative markers and QTL alleles. Thus it is important to identify two inbred lines which are informative, and to carefully design the mapping population.

While the typical mapping population in outcrossing species is the use of sibs or other close relatives (Xu, 1995), a great variety of designs are possible with inbred line crosses. A standard F_2 design can be used, as can a backcross design where the F_1 individual is backcrossed to a parent from one of the original inbred lines ($F_1 \times P_1$ or $F_1 \times P_2$).

Recombinant inbred lines (RILs) are produced by selfing many generations from the F_1 parents. Likewise it may be possible to form doubled haploids (DHs) in some species by taking gametes from F_1 individuals and doubling the chromosome number, creating diploid individuals that are completely homozygous at all loci for QTL mapping. Near isogenic lines (NILs) are produced by backcrossing different F_1 individuals to the same original parent for introgression of the target chromosome region and these NILs are especially useful in fine QTL mapping.

2.3.1.1. F_2 and Backcross Populations

F_2 and BC populations are widely used in QTL mapping. The main reason is that these populations can be produced easily in almost every plant species. Interspecies F_2 or BC populations can even be used in mapping QTLs. This is especially valuable in identifying useful exotic germplasm in crop improvement. The problem of F_2 and BC

populations is their ephemeral property for long-term evaluation, unless plants can be cloned. Part of this problem is resolved through the $F_{2:3}$ generation for future evaluation.

2.3.1.2. Recombinant Inbred Lines (RILs)

Since RILs are formed by inbreeding, further rounds of recombination occur while lines are being inbred to fixation, The frequency of recombinant gametes in the RILs are increased compared with that of F_2 population. Because of this expansion in map distance, RILs have an advantage over conventional segregating populations, such as F_2 or BC populations in fine mapping, but a disadvantage in coarse mapping of QTLs (Darvasi and Soller, 1994).

Another major advantage of RILs is that once the considerable work to generate a set of these lines is done, essentially any character of interest can be examined for marker-QTL association. Hence lines generated to examine one set of characters are potentially very powerful for examining other different characters, and new data are added continually to the preexisting map. RILs also offer a particular easy approach for measuring the genotype-environment interaction associated with particular QTLs, since the same RILs can be planted over different sets of environments (Burr *et al.*, 1988).

2.3.1.3. Doubled-Haploid Lines (DHL)

Like the RILs used in QTL mapping, a related approach is the use of doubled-haploid lines (DH), where haploid gametes are treated to double the chromosome

number, produce completely homozygous individuals (Hayes *et al.*, 1993). Doubled-haploid lines experience only a single generation of recombination so no correction of recombinant frequency between QTL and marker is required. A major problem with doubled-haploid lines is that they occur only in species such as barley (Hayes *et al.*, 1993).

2.3.1.4. Near Isogenic Lines (NILs)

Most NILs have been developed by introgression. This consists of many generations of backcrossing the genes of interest from the non-recurrent parents to a recurrent parent. NILs are almost identical in genetic background except the genome region around the target genes. Brewbaker (1995) developed a set of NILs on the same genetic background inbred Hi27 in Hawaii, including 120 morphological markers scattered throughout the ten chromosomes of corn.

Unlike other mapping populations in QTL mapping, NILs are useful in identifying tightly linked markers in QTL mapping. Accurate localization of QTLs can be obtained using NILs, these NILs will eliminate the majority of the genetic variance and will make it possible to dissect the remaining unlinked markers while in detecting the linked markers associated with QTLs (Paterson *et al.*, 1991).

2.3.2. Selective Genotyping and Bulked Segregation Analysis

Selective genotyping and bulked segregation analysis both refer to the selection of the extreme phenotypes for genotyping, and mainly for increase the efficiency for mapping

program.

2.3.2.1. Selective Genotyping

One important strategy that can significantly increase the power of an experimental design in mapping QTLs is selective genotyping. This strategy is to select two subsets of the two extreme phenotypes and then genotype these individuals with molecular markers. The advantages of this approach are less effort and lower cost (Lander and Botstein,1989; Darvasi and Soller,1992). The basis of this approach is that much of the linkage information can be reflected from individuals with extreme phenotypes. Darvasi and Soller (1992) proposed that the scope of the selective genotyping was about 25 percent of the whole populations for both extreme phenotypes.

While selective genotyping offers increased power in mapping QTLs, it also produces biased estimates of the QTL effect (Lander and Botstein 1989, Darvasi and Soller 1992).

2.3.2.2. Bulked Segregant Analysis

A variant of selective genotyping is bulked segregant analysis or pooled-sample approach (Michelmore *et al.*, 1991). The idea of this approach is to select both extreme phenotypes based on trait value in a segregation population and then to combine these selective phenotypes into groups (bulks, pools). DNA from each bulk is screened en masse for a number of markers. Unlinked markers will be randomly distributed across each bulk,

with linked marker(s) present only in one bulk and the alternative allele present only in the other bulk (Darvasi and Soller, 1994).

Bulked segregation analysis is straightforward, and allows for rapid analysis in identifying QTLs. Paran *et al.* (1991, 1993) used bulked segregant analysis to obtain RFLP and RAPD markers linked to downy mildew resistance in lettuce. McMullen *et al.* (1995) identified three major QTLs, ws1, ws2, and ws3, conferring resistance to wheat streak mosaic virus in maize using this bulked segregant analysis. Chague *et al.* (1996) identified and mapped Sw-5 gene for resistance to tomato spotted wilt virus (TSWV) in tomato using this method.

Bulked segregant analysis can also be used to locate molecular markers in defined chromosome. Any genome region of interest that has been previously mapped by molecular markers can thus be targeted rapidly with new markers. This may be especially useful in trying to fill in gaps or identifying large numbers of molecular markers in a specific chromosomal region.

2.3.3. Progeny Testing

Another powerful experiment design in mapping QTLs is by progeny testing. Its main purpose is to dissect the environmental influence by testing genotyped individuals in different environments and using mean trait values from different environments in substitute of a single trait value from only one environment. Repeated progeny testing also allow the measurement of genotype - environment interactions that are especially

important in the breeding application. Both RILs and DH populations can be used practically in progeny testing (Knapp *et al.*, 1991).

2.4. Statistical Analysis for Mapping QTLs

How to detect an association between polymorphic markers and quantitative trait phenotypes depends on greatly the statistical method. The simple method in identifying this association is by using a single marker (Weller, 1986). Interval mapping proposed by Lander and Botstein (1989) use flanker markers instead of single markers in identifying association of markers and QTLs. Composite interval mapping, which combines multiple regression with interval mapping, is more efficient in QTL mapping because it excludes the influence of the other markers in the procedure of interval mapping (Zeng 1994, Jansen 1994).

2.4.1. Single Marker

There are two different approaches in single marker analysis. One approach is the linear model test, including t-test, ANOVA, and regression. Another approach is by using the maximum likelihood method (Knapp *et al.*, 1990; Arus *et al.*, 1993).

The linear model test in detecting QTL is to compare the phenotypic means of different marker genotypes. When only two marker genotypes are being compared, t-test for significant difference in means provides a simple but effective test for the presence of a linked QTL. QTL effects can also be estimated from the analysis of marker genotype means.

One apparent disadvantage with this simple t-test based on differences between homozygote marker means is that heterozygous markers are ignored. ANOVA or regression with the consideration of all marker genotypes can avoid this problem. The mathematic model is as follows:

$$Z = m + b_1X_1 + b_2X_2 + b_3X_3 + e$$

In this formula, Z is the dependent variable (quantitative trait), m is the mean value of the trait, the three independent variables X_1, X_2, X_3 denote the three marker genotypes, b_1, b_2, and b_3 are coefficients of the three marker genotypes, and e is the residual error.

Another approach in identifying QTLs by single marker analysis is the maximum likelihood method. In this approach, detection of the association between QTLs and markers depends on the maximum likelihood ratios as follow:

$$\Lambda(z) = -2\{\ln[\max l_r(z)]-\ln[\max l(z)]\}$$

In this formula, max l(z) is the product of each maximum likelihood for the full set of data, while max l_r(z) is a restricted max l(z) under the null hypothesis of no segregating QTL. The resulting test statistic is chi-square distributed with n-r degree of freedom (n is number of the all characters in the full model while r is numbers of the specified characters in the restricted model). Identification of QTL is often displayed graphically through the use of likelihood maps, which plot the likelihood ratio statistic as a function of map position. Maximum likelihood methods are powerful in single marker analysis (Weller, 1986). One of the disadvantages of this method is that it does not yield meaningful results for minor QTLs unless a large number of individuals are scored.

The disadvantages of single marker analysis are: (1) estimations of QTL effects are biased by the recombination frequency between the marker and the QTL, (2) if several QTLs were associated with marker locus, or one QTL was associated with several markers, the single marker analysis can not separate each individual QTL with a specific marker, and (3) if the heritability of the trait is low, phenotypic values of individual plants will have a large environmental error component. The best way to increase the precision of QTL analysis is thus look at many progeny, especially in different environments.

2.4.2. Flanking Markers

The use of flanking markers together with the maximum likelihood method in QTL mapping (interval mapping via maximum likelihood) has been proposed by Lander and Botstein (1989) and Knapp *et al.* (1990) as a means of overcoming some of the limitations of single marker analysis. Haley and Knott (1992) recommended a regression approach in interval mapping, very similar to maximum likelihood method. Estimations of QTL effects and positions are much more precise using flanking markers instead of using single markers. Interval mapping is probably the most familiar method of QTL mapping at present.

2.4.2.1 Interval Mapping via Maximum Likelihood

Lander and Botstein (1989) and Knapp *et al.* (1990) have developed a maximum likelihood method for mapping QTL using flanking markers. This method is similar to the

maximum likelihood method described above in single marker analysis is based on flanking markers instead of single markers. It assumes that phenotypes are normally distributed with common variance in each QTL phenotype. The resulting likelihood functions are mixture models, and additional assumption of using flanking markers is that no double crossover occurs between flanking markers, which in fact is very rare (Knapp *et al.* 1990). Maximum likelihood involves searching for QTL parameters that give the best approximation for quantitative trait distributions that are observed for each marker class. The evidence of the presence of a QTL is based on the maximum likelihood ratio (LOD score) tests:

$$LOD = \log_{10}[L(a, b, \sigma^2)/L(u_0, 0, \sigma_0^2)]$$

Where likelihood function L are derived from the following model:

$$P_i = a + bg_i + e$$

In this formula, P_i and g_i are phenotype and genotype for the ith individual, a and b are phenotype mean and coefficient, and *e* is error term. $L(a, b, \sigma^2)$ is likelihood function for all individuals while $L(u_0, 0, \sigma_0^2)$ is a restricted likelihood function of $L(a, b, \sigma^2)$ under the assumption that no QTL effect occurs($a=u_0$, $b=0$ and $\sigma^2 = \sigma_0^2$). The likelihood map can be constructed by plotting the LOD scores as a function of interval map distance. The peak of the likelihood map corresponds to the maximum likelihood estimate of QTL position within that interval. The likelihood map for an entire chromosome can be constructed by combining each successive interval.

The power of the maximum likelihood method using flanking markers in mapping

QTL has been examined by Lander and Botstein (1989), Van Ooijen (1992), and Darvasi *et al.* (1993) and has been confirmed by many experimental results (Tanksley, 1993). Shortcomings of this approach still exist (Haley and Knott, 1992; Zeng, 1994). The main limitation is that the identified QTLs by using this method may be confounded by the unlinked markers outside the flanking region.

2.4.2.2. Interval Mapping via Regression

Interval mapping by regression was developed mainly as a simplification for the maximum likelihood method (Haley and Knott, 1992, Martinez and Curnow, 1992). The phenotypes are regressed on QTL genotypes estimated from the nearest flanking markers. Haley and Knott (1992) computed the regression at each interval with the largest r^2 taken as the estimate of QTL position in the interval and make a r^2 plot across the whole chromosome. Interval mapping via regression method is actually a simplification of interval mapping via maximum likelihood method, and results from the two methods are almost identical (Haley and Knott, 1992)

2.4.3. Multiple Markers

Using all the markers at the same time instead of using the two flanking markers can alleviate part of the limitation of interval mapping. The most popular method of using multiple markers is composite interval mapping, which is a combination of interval mapping and multiple regression (Zeng, 1993, 1994; Jansen, 1993, 1994, 1996). Another approach in using multiple markers is to identify the epistatic interactions.

2.4.3.1. Composite Interval Mapping

Composite interval mapping is a combination of interval mapping and multiple regression (Zeng, 1993, 1994; Jansen, 1993, 1994, 1996) and designed mainly to increase the precision of interval mapping by multiple regression analysis of the markers outside the region of flanking markers. Theoretically, it should be more powerful and precise because it considers multiple markers outside interval markers as a cofactor in the interval mapping process.

2.4.3.2. Epistasis

Epistatic interaction among genes can play an important role in plant phenotypic expression and evolution (Li *et al.*, 1997). Detection and estimation of epistasis by traditional biometrical methods can be difficult (Lander and Botstein, 1989). Information from molecular marker studies provide a direct method to estimate epistatic interactions among QTLs (Cheverud and Routman, 1995; Li *et al.*, 1997).

2.5. Threshold Value in QTL Mapping

A problem common to all the above methods is how to determine the appropriate significance thresholds (usually LOD score or likelihood ratios) for the detection of any QTL. The LOD threshold value is related to both the chromosome size and the marker density in the chromosome (Lander and Botstein, 1989). The LOD score threshold for avoiding a false positive with 0.95 probability when testing 60 flanking markers in 1200

cM was estimated to be about 2.4 (Lander and Botstein, 1989). This threshold value was widely used to identify QTL in interval mapping using F_2 population.

Some promising developments in computer-intensive statistical methods based on the power of electronic computation have been applied to QTL mapping. Permutation proved powerful in establishing the threshold value in interval mapping, and is a method of establishing significance without making assumptions about the data (Churchill and Doerge, 1994; Doerge and Churchill, 1996). Visscher *et al.*, (1996) proved the feasibility of bootstrap method in determining approximate confidence intervals for the mapping of QTLs using simulation result. Bayesian analysis, implemented with a Markov Chain Monte Carlo (MCMC) method, was also tested as a reasonable method in the determination of the threshold value by both data simulations and experiment results (Hoeschele and VanRanden, 1993; Satagopan *et al.*, 1996).

The first step in permutation is to scramble the relationship between quantitative trait observations and marker genotypes, then perform interval mapping with the permuted data and repeat these two steps many times (e.g. 1000) to choose a threshold value. This procedure has been incorporated into the computer program, such as QTL cartographer (Basten *et al*, 1997).

2.6. Marker Assisted Selection and Marker Based Cloning

Since QTLs mapping initiated last decade became feasible, there has been an explosion in mapping QTLs conferring grain yield, grain nutrition values, disease and pest

resistance and agronomic and physiological traits in almost every economic crop (Staub et al., 1996; Paterson, 1997).

Plant breeders and plant geneticists seek more efficient methods for crop improvement. Among these methods, marker assisted selection and marker based cloning offer opportunities for more efficient exploration and utilization of existing and exotic germplasm. Theoretical research shows great potential in the use of marker-based methods and marker-based cloning in crop improvement. Implementation of these methods in actual breeding practice will be a major challenge for breeders in the next century.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Plant Materials

The development of recombinant inbred lines (RILs) from the tropical maize single crosses and of sublines of the parents at Waimanalo Research Station was described by Moon (1995). A total of 110 RILs were developed from the cross Hi34 x TZi17 by single seed descent procedure and called set I (Moon *et al.*, 1998). Hi34 is a tropical yellow flint inbred derived from Antigua 2D and developed in Hawaii. TZi17 is a tropical white flint inbred derived from the cross RppSR x Oh43 and developed at IITA, Nigeria. The cross Hi34 x TZi17 was made in Hawaii in 1986. Two hundred F_2 seeds from several ears were selected randomly and planted in Spring 1990. F_3 seeds from each harvested ear were planted ear to row, and one self-pollinated ear from each row was selected to advance the lines to the next generation. This single seed descent was practiced for six cycles of selfing to the F_7 generation in the absence of selection (Moon *et al.*, 1999). Ten plants from each F_7 inbred were sib-pollinated to supply seed for future experiments.

3.2. Detecting Major QTLs in RILs

The normal distribution curve method (Brewbaker, 1995) and the maximum likelihood method were applied to detect major QTLs conferring disease and insect resistance segregating in the population of RILs.

3.2.1. Normal Distribution Curve Method

The formula for describing a normal frequency distribution is:

$$f(z, \mu, \sigma^2) = \frac{n}{\sigma\sqrt{2\pi}} e^{-(z-\mu)^2/2\sigma^2}$$

Where f is the frequency of occurrence of any given variant, z is any given variant, n is the number of individuals in the population, μ is the population mean and σ is the population standard deviation. The normal distribution curve describing the frequency of occurrence of variants can be plotted by the calculation of just the two parameters, μ and σ.

Brewbaker (1995) developed a normal distribution curve method for identifying major QTLs using spreadsheets (Quattro, Excel). Based on the parental means and variances, the distributions for monogenic, digenic, and polygenic segregation in RILs can be predicted. Goodness of fit for observed data can be tested using chi-square and least squares estimates.

3.2.2. Maximum Likelihood Method

Suppose n observed phenotypic values ($z_1 \ldots z_n$) of specific RILs are from an

$$l(z) = (2\pi\sigma^2)^{-n/2} \exp[-\Sigma \frac{(z-\mu)^2}{2\sigma^2}]$$

underlying normal distribution with unknown mean μ and variance σ. The resulting likelihood function $l(z)$ is then defined as follow:

Given a likelihood function, likelihood ratio tests provide a procedure for testing a very wide variety of hypotheses about the unknown parameters:

$$\Lambda(z) = -2\{\ln[\max l_r(z)]-\ln[\max l(z)]\}$$

Where $l_r(z)$ is the likelihood function evaluated at the maximum likelihood estimate for the restricted model, the simplest of which is a single normal distribution with unknown mean and variance. Maximum likelihood estimate of the unknown mean and variance is the sample mean and sample variance. While $l(z)$ is the full model under the hypothesis of major QTL segregated in the population. In case of a major QTL segregating in the RILs, the distribution for $z_1 \ldots z_n$ is a mixture model and likelihood function $l(z)$is as follow:

$$l(z) = \frac{1}{2}[(2\pi\sigma^2)^{-n/2} e^{-(z-\mu_{QQ})^2/2\sigma^2}] + \frac{1}{2}[(2\pi\sigma^2)^{-n/2} e^{-(z-\mu_{qq})^2/2\sigma^2}]$$

Where μ_{QQ} and μ_{qq} are means of the two genotypes (QQ and qq) segregating in the RILs. The test statistic is a chi-square test (Jiang *et al.*, 1994; Weir, 1996).

3.3. RFLP Analysis

3.3.1. DNA Extraction

RFLP analyses follow the following steps: DNA extraction, restriction enzyme digestion and agarose electrophoresis, southern transfer, probe preparation, and hybridization (Hoisington *et al.*, 1993) in the present study of set I. Young seedling leaves from the two parental lines and all RILs were frozen with liquid nitrogen, then lyophilized for 5 days. The lyophilized samples were ground to a fine powder with a mechanical mill and ground samples were stored tightly capped at -20^{0}C.

Total maize genomic DNA was prepared using the method of Saghai-Maroof *et al.* (1984). Samples of 0.3-0.4g of ground, lyophilized powder were incubated for 60-90 minutes in 9 ml of warm (65^{0}c) CTAB (mixed alkyltrimethyl-ammonium bromide) extraction buffer (1% CTAB, 0.1M tris pH 7.5, 0.7M NaCI. 10mM EDTA pH 8.0, and 140mM β-mercaptoethanol). An extractant solution of 4.5 ml chloroform/octanol (24:1) was added after incubation. DNA was treated with RNAase A (50 µl of 10mg/ml) just before iospropanol precipitation. The precipitated DNA was removed with a glass hook and transferred to a 5 ml tube containing 1 ml of TE (10 mM Tris pH 8.0, 1 mm EDTA) and extracted with phenol followed by a chloroform. The DNA was brought to 0.25 M NaCl and precipitated with 2.5 volumes of cold ethanol. Spooled DNA was washed in 76% ethanol, 0.2 M sodium acetate, followed by 76% ethanol, 10 mM ammonium acetate and re-suspended in TE at a final concentration of 0.5 µg/µl. DNA was stored at 4^{0}C for short times and at -20^{0}C for longer periods, respectively.

3.3.2. Restriction Enzyme Digestion and Agarose Electrophoresis

Maize genomic DNA (20μg) was digested in a total volume of 300 μl solution with 2.5 units of restriction enzymes/μg DNA for 4 hours at 37^0C to insure complete digestion. The reaction was stopped by adding 16 μl of 5 M NaCl and EcoRI (or HindIII). DNA was precipitated by adding 750 μl of ethanol and re-suspended in 40 μl TE, which allowed DNA to be loaded into the agarose gels at a concentration of 10 μg/lane.

Agarose gels (0.7%) were run for about 14-16 hours until the bromophenol blue tracking dye migrated 5.5cm. Gel dimensions were 20cm x 25 cm, which allowed four sets of combs with 25 or 30 wells to be used on a single gel. After electrophoresis, gels were stained in ethidium bromide (1 μg/ml) for 20 minutes. The gels were then rinsed in dH_2O for 20 minutes and photographed.

3.3.3. Southern Transfer

Gel were denatured for 30 minutes in 0.4N NaOH, 0.6M NaCl, followed by neutralization in three volumes of 0.5M Tris-pH 7.5, 1.5M NaCl for 40 minutes. DNA was then blotted onto nylon membranes (MSI Magnagraph, Fisher Scientific) with transfer buffer (25 mM $NaPO_4$ pH 6.5) using a method modified after Southern (1975) which utilized cellulose sponges for wicking. After blotting overnight (6-18 hours) with one change of paper towels, membranes were immediately placed in 2X SSC (1X SSC, 150 mM NaCl, 15mM Sodium Citrate) for washing 15 minutes, dried, and UV-Stratalinked to bind the DNA to the membranes according to the manufacturer's recommendations (Stratagen, San Diego, CA). Membranes were then baked at 92^0C for 2-4 hours.

3.3.4. Probe Preparation

Plasmids were isolated from 10ml cultures. Insertions were obtained by digesting 20 µg of each plasmid with the appropriate enzyme and electrophoresed in TAE (40 mM Tris, 5mM EDTA pH 8.0). Gels agarose plugs containing the insert in TE were diluted to a final concentration of 10ng/µl for incorporation of Digoxigenin-dUTP.

Incorporation of Digoxigenin-dUTP was done using 50 ng of probe insert DNA and 5.0µl of Digoxigenin - dUTP for a 250 cm^2 membrane (Hoisington *et al.*, 1994).

3.3.5. Hybridization

The membranes were prehybridized at 65^0C in a buffer which consisted of 5X SSC, 50 mM Tris-pH 8.0, 0.2% SDS, 10mM EDTA-pH 8.0, 0.1 mg/ml denatured Sonicated Salmon DNA and 1X Denhardt's solution (0.02g Ficoll 400, 0.2g polyvinylpyrollidane 4000, 0.02g bovine serum albumin, fraction V). After 4 hours, the prehybridization solution was removed and replaced with hybridization buffer (3.0ml/250cm^2) which contained 10% Dextran Sulfate and denatured probe. Membranes were hybridized overnight at 65^0C. Membranes were then washed 2 x 5 min in 0.15X SSC, 0.5% SDS followed 3 x 15 min wash in 0.15X SSC, 0.1% SDS at 65^0C. After washing, membranes were exposed to X-ray film with an intensifying screen at -80^0C for 1-6 days depending on the intensity of the signal. Autoradiographs were obtained by developing films in a Kodak X-OMAT M20 processor.

3.4. Simple Sequence Repeats (SSRs)

3.4.1. DNA Extraction

The SSR analyses followed three steps: DNA extraction, PCR, and electrophoresis. DNA extraction was from young leaves of the RILs and their two parental lines. The protocol for DNA extraction was the same as that of RFLP analysis, with a slight difference in that the amount of DNA required in SSR analysis (about 50 ng) was much less than in RFLP analysis (about 5μg).

3.4.2. PCR and Electrophoresis

PCR was performed in a 20ml volume containing 25ng of DNA, 5 pmol of each primer, 200 μM of each dNTP, 90 mM Tris-HCl (pH 9.0), 20 mM $(NH_4)SO_4$, 2.5mM $MgCl_2$, and 0.75 U Taq polymerase (Perkin Elmer Cetus, Norwalk, Conn. USA). Amplifications were performed using a Perkin Elmer 9600 Thermal Cycler with the following conditions: 93^0C for 2 minutes (1 cycle), 93^0C for 1 minute, 56^0C for two minute, 72^0C for 2 minute (30 cycles), and 72^0C for 5 minutes (1 cycle). An equal volume of stop solution (98% deionized formamide, 2 mM EDTA, 0.05% bromophenol blue, plus 0.05% Xylene Cyand) was added to PCR products and heated for 3 minutes at 95^0C. A 3 ml aliquots of each reaction mixture were analyzed by 6% Metaphor : Seakem agarose electrophoresis.

3.5. Linkage analysis and QTLs mapping

Advances in computer technology have been essential to programs in the construction of marker maps and QTL mapping. The single factor analysis in this experiment was conducted using the Proc GLM procedure in SAS (SAS Institute Inc., Cary. NC).

The most widely-used genetic mapping software is MAPMAKER (Lander *et al.*,1989). MAPMAKER (MAPMAKER/EXP 3.0) is based on the theory of interval mapping via a maximum likelihood method. An important concept in this program is the LOD score, the "log of the odds-ratio". Linkage was declared when LOD value exceeded 3.0, with a maximum recombination frequency of 0.40. The Haldane mapping function was used.

Recently many computer programs have been developed for QTL mapping. Almost all of the new developed programs are based on the theory of composite interval mapping. *QTL Cartographer*, one of the new QTL mapping programs, was used in this experiment (Basten *et al.*, 1997). This program implements the simultaneous mapping of multiple traits using the interval and composite interval method. It includes a dynamic algorithm that allows a host of statistical models to be fitted and compared, including various gene actions, QTL-environment interactions, pleiotropic effects and close linkage.

In addition to the identification of the pairwise interactions by use of SAS GLM, several computer programs have been developed to dissect the epistatic interactions among QTLs (Holland, 1998; Chase *et al.*, 1997; Wang *et al.*, 1998). Epistat identifies

and tests interactions between pairs of quantitative trait loci, and is based on the theory of maximum likelihood methods together with Monte Carlo simulations (Chase *et al.*, 1997).

CHAPTER FOUR

MAPPING OF QUANTITATIVE TRAIT LOCI CONFERRING RESISTANCE TO MAIZE STREAK VIRUS

Abstract

Maize streak virus (MSV) causes a major disease of maize in Africa. TZi17, a tropical maize inbred with general resistance to MSV, was crossed to a susceptible tropical maize inbred, Hi34, and 110 recombinant inbred lines (RILs) were produced by single seed descent without selection. The RILs were genotyped with 116 restriction fragment length polymorphisms (RFLPs) and 4 simple sequence repeats (SSRs). The same population had been evaluated for resistance to MSV under natural infections in winter 1992 and winter 1993 at IITA, Nigeria. RFLP markers were shown to be linked to a major quantitative trait locus (QTL) on chromosome 1 conferring resistance to MSV through the use of composite interval mapping. The interval between RFLP marker asg30 and umc167 explained about 29.6% of the phenotypic variance with a peak LOD score of 6.0. A minor QTL for resistance to MSV was also identified and mapped on chromosome 9, with a peak LOD score of 3.0 that explained about 5.9% of the phenotypic variance.

4.1. Introduction

Maize streak virus (MSV), transmitted by *Cicadulina* spp. leafhoppers, is widely distributed and causes a major disease of maize, especially in Africa (Efron *et al.*, 1989; Kim *et al.*, 1989). Yield losses due to MSV range up to 100% when epidemics occur. The host range of MSV is wide among economic crops and includes maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), sorghum (*Sorghum bicolor* (L.) Moench), sugarcane (*Saccharum officinarum* L.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), rye (*Secale cereale* L.) and rice (*Oriza sativa* L.). Some wild grasses also act as alternative hosts, but maize is a preferred host. Symptoms in maize include chlorotic, almost circular spots with a diameter of 0.5-2 mm in the youngest leaves. Prominent white chlorotic streaking along the veins develops on older leaves and plants became stunted. The potential threat of MSV to maize production is worldwide especially in the tropical lowlands, and most maize varieties are highly susceptible (Brewbaker *et al.*, 1991).

Cultural practices such as timely planting and crop rotation can reduce the losses to MSV. However, the most effective and economic control of MSV is through the development of resistant varieties (Kim *et al.*, 1989). Maize breeders at the International Institute of Tropical Agriculture (IITA) in Nigeria and the International Maize and Wheat Improvement Center (CIMMYT) at their Zimbabwe station have made efforts to develop MSV resistant varieties and populations, mainly through backcross conversion (Barrow *et al.*, 1992; Kim *et al.*, 1989).

Extensive screening of a wide range of materials in South Africa identified genetic resistance resources such as the cultivar Peruvian Yellow (Fielding, 1933), Arkell's Hickory (Rose, 1936), and Tropical Zea Yellow or TZY (Soto *et al.*, 1982). Storey and Howland (1967) concluded that resistance to MSV was monogenic with incomplete dominance by studying the segregation ratios in inbred lines from the cross 'Peruvian Yellow' x 'Arkell's Hickory'. Resistance to MSV in the cultivar 'Tropical Zea Yellow' was transferred to a highly productive inbred, IB32 (Soto *et al.* 1982) that was used as an MSV- resistance donor at IITA. Kim *et al.* (1989) reported that resistance to MSV in IB32 was controlled quantitatively with relatively small numbers of genes involved through generation mean analysis. Narrow and broad sense heritability values were estimated at 55% and 83% respectively.

Other sources of MSV resistance included Mexican inbreds Mex37-5, Urg54 and Gto29-29A-5-4, Rhodesian inbred 3NA, Caribbean variety 'Yellow Bounty' and the Reunion varieties 'Revolution' and 'IRAT 297' (Gorter, 1959; Rodier *et al.*, 1995). Rodier *et al.* (1995) reported that one major dominant gene and several other minor genes were responsible for the resistance of IRAT297 to MSV by generation mean analysis. Kyetere et al. (1995) mapped a gene on chromosome 1 for MSV tolerance from a Hawaiian recombinant inbred lines (RIL) population based on TZi4, an inbred derived from Nigerian streak-resistant population TZSR crossed with Hi34 from Hawaii. Moon *et al.* (1998) concluded that a single major gene could be responsible for resistance to MSV through

analysis of three RILs using a spreadsheet-based normal probability method and maximum likelihood method.

Molecular markers like RFLPs allow the resolution of quantitative traits into Mendelian factors referred to as quantitative trait loci, or QTLs (Paterson *et al.*, 1988). Construction of molecular marker maps and QTL mapping provides information on both the genome regions and genetic effect of the QTLs involved in different traits. Marker assisted selection and marker based cloning can be adopted following identification and characterization of appropriate QTLs.

In this study, we used 110 RILs derived through single seed descent from the cross of Hi34 and TZi17 to map QTLs conferring resistance to MSV. The objectives of this study were to determine the genome positions of QTLs conferring resistance to MSV and to estimate the genetic effect of the QTLs.

4.2. Materials and Methods

4.2.1. MSV Screening

One hundred and ten RILs and the two parents (Hi34 and TZi17) were planted by Dr. Soon Kwon Kim for MSV resistance evaluations in the winter season of 1992 and 1993 in Ibadan, IITA, Nigeria. Hi34 is a tropical yellow flint inbred derived from Antigua 2D and developed in Hawaii. TZi17 is a tropical white flint inbred derived from the cross RppSR x Oh43 and developed at IITA, Nigeria. Both trials were planted in single row

plots 5 m long with 0.75 m between-row spacing with about 20 plants per row without replication. MSV screening was under natural infection due to the year-round cultivation and continuous epibiotics of MSV at this location. Ten RILs failed to germinate in the winter 1993 trials. The MSV ratings were rated on the first 10 plants of the whole plot based on a 1-9 scale as follows (Kim *et al.*, 1989):

1 = very few streak symptoms on lower leaves (highly resistant)

3 = light streak symptoms on most leaves below ear with few symptoms above ears

5 = moderate streak symptoms on most leaves, with some host tolerance

7 = abundant symptoms on 60% of leaf area and plant growth suppressed

9 = severe streak symptom on 75% of leaf area, no ears formed, plant growth severely suppressed or plant dead.

4.2.2. RFLP and SSR Assays

The 110 RILs and the two parents (Hi34 and TZi17) were planted in the winter season 1996 at the Poza Rica Station of CIMMYT in Vera Cruz, Mexico. The trial was planted in a two-replication randomized complete block design with the purpose of DNA extraction and field evaluation of fall armyworm resistance for another experiment. Leaf tissue from 10 field-grown plants per line was bulked, lyophilized and ground to a fine powder for DNA extraction to determine the genotype of the two parents and the RILs. DNA extraction followed the modified CTAB procedure based on the method of Saghai

Maroof *et al.* (1984). RFLP probes were chosen mainly from the collection of the University of Missouri (UMC), California State University (CSU), Brookhaven National Laboratory (BNL), and Native Plants Inc. (NPI). Two hundred and nine RFLP probes with two enzymes (EcoRI and HindIII) were used to screen the two parents. One hundred and sixteen RFLP probes were selected to genotype the RILs based upon the results from parental screening. Four SSRs (*mag1f03, phi22, phi 93, phi115*) showing polymorphism between the two parents were also selected to genotype the RILs following the protocol from CIMMYT AMG Laboratory (Hoisington *et al.*, 1994). The segregation of alleles for both RFLP and SSR markers were checked against the expected 1:1 ratio for RILs by a chi square test.

4.2.3. Linkage Analysis and QTL Mapping

MAPMAKER/EXP 3.0 (Lander *et al.*, 1987, Lincoln *et al.*, 1992) was used to make the linkage map from a total of 107 RFLP and four SSR markers. Data were entered using "data type ri self" format. Linkage was declared when LOD (log 10 of the likelihood ratio) value exceeded 3.0, with a maximum recombination frequency of 0.40. The Haldane mapping function was used (Lincoln *et al.*, 1992).

Single-factor analysis of variance for identifying marker-QTL linkage was conducted using the GLM procedure in SAS (SAS Institute, 1989). Two-factor analyses of variance were also computed for each possible pair of loci to determine main effects of the two loci plus their interaction. Pre-selection techniques were used to reduce the

number of factors to be considered and main effects of loci were considered for model building if they were significant at $P \leq 0.05$. QTL Cartographer version 1.12e (Basten *et al.*, 1997) was used to map the putative QTLs through interval mapping (Lander and Botstein, 1989) and composite interval mapping (Zeng, 1993, 1994). Forward and backward stepwise regression (FB) was selected for the identification of cofactors for the composite interval mapping. The significance threshold used for QTL detection with the interval mapping and the composite interval mapping was determined from 1000 permutation test (Churchill and Doerge, 1994) by using QTL Cartographer software.

4.3. Results

4.3.1 Phenotypic Data

TZi17 averaged 2.24 and Hi34 averaged 7.04 on 1-9 scale for the winter 1992 trials in response to the MSV infestation (Table 4.1). Data were taken by Dr. Soon Kwon Kim and staff at IITA, Nigeria (Moon, 1995). In the winter 1993 trial, TZi17 averaged 2.20 and Hi34 averaged 7.20. Highly significant differences (at 1% level) were observed among the RILs in both trials. The correlation coefficient for MSV resistance in the two trials was 0.597 ($P<0.01$).

The distribution of the RILs for resistance to MSV averaging the two trials) indicated that the observed data were not significantly different from the expected 1:1 segregation following the normal distribution methods (Figure 4.1) of Brewbaker (1996).

Table 4.1. Means and standard deviations of parents Hi34 (susceptible) and TZi17 (resistance) and their RIL population for MSV scores.

	MSV Scores*		
Parameter	1992	1993	Average
	---------- 1-9 scale----------		
Hi34	7.04 ± 1.11	7.20 ± 0.69	7.12 ± 0.93
TZi17	2.24 ± 0.51	2.20 ± 0.49	2.22 ± 0.50
RIL	3.73 ± 2.03	4.71 ± 2.54	4.02 ± 1.72
Range of RILs	2.0 - 8.0	1.0 - 9.0	1.5 - 7.5

* Scale: 1=resistant to 9 = susceptible.

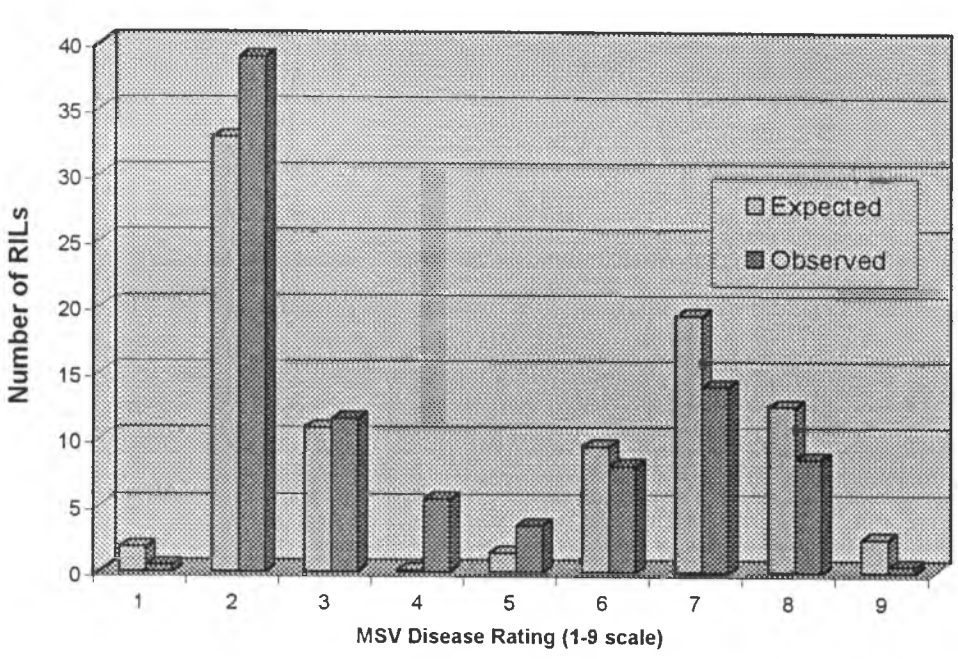

Figure 4.1. Mean disease rating of 100 RILs derived from Hi34 xTzi17 for resistance to MSV at IITA, Nigeria

The existence of a major gene for resistance to the MSV was also detected in the RILs by the maximum likelihood method, with a likelihood ratio of 14.36 ($P<0.01$) based on the assumption of the presence of single major gene (Appendix 3).

4.3.2. Genotypic Data

One hundred and sixteen RFLP markers (Figure 4.2) and four SSR markers (Figure 4.3) showed polymorphism between the two parents, and were chosen to genotype the RILs. RILs showing hybrid bands and non-parental bands were coded as missing data. Among the 116 RFLP markers, 91 RFLP markers fit the 1:1 segregation ratio, 15 RFLP markers skewed to Hi34 allele (66.6%) and the other 10 RFLP markers skewed to TZi17 allele (65.2%). All four SSR markers fit the 1:1 segregation ratio.

The overall averages of the Hi34 alleles and TZi17 alleles were 50.2% (Figure 4.4) and 49.8% (Figure 4.5) in the RILs, respectively. This indicated that both parents contributed evenly in the development of the RILs.

4.3.3. Map Construction

Seventy RFLP markers were selected with single copy in the linkage groups and without significant distortion from 1:1 ratio. The primary linkage groups were formed from these markers based on the UMC RFLP map (http://www.agron.missouri.edu). By the 'assign' command in MAPMAKER/EXP 3.0 program, another 34 RFLP markers and three SSR markers were integrated into the primary linkage groups. The orders of these

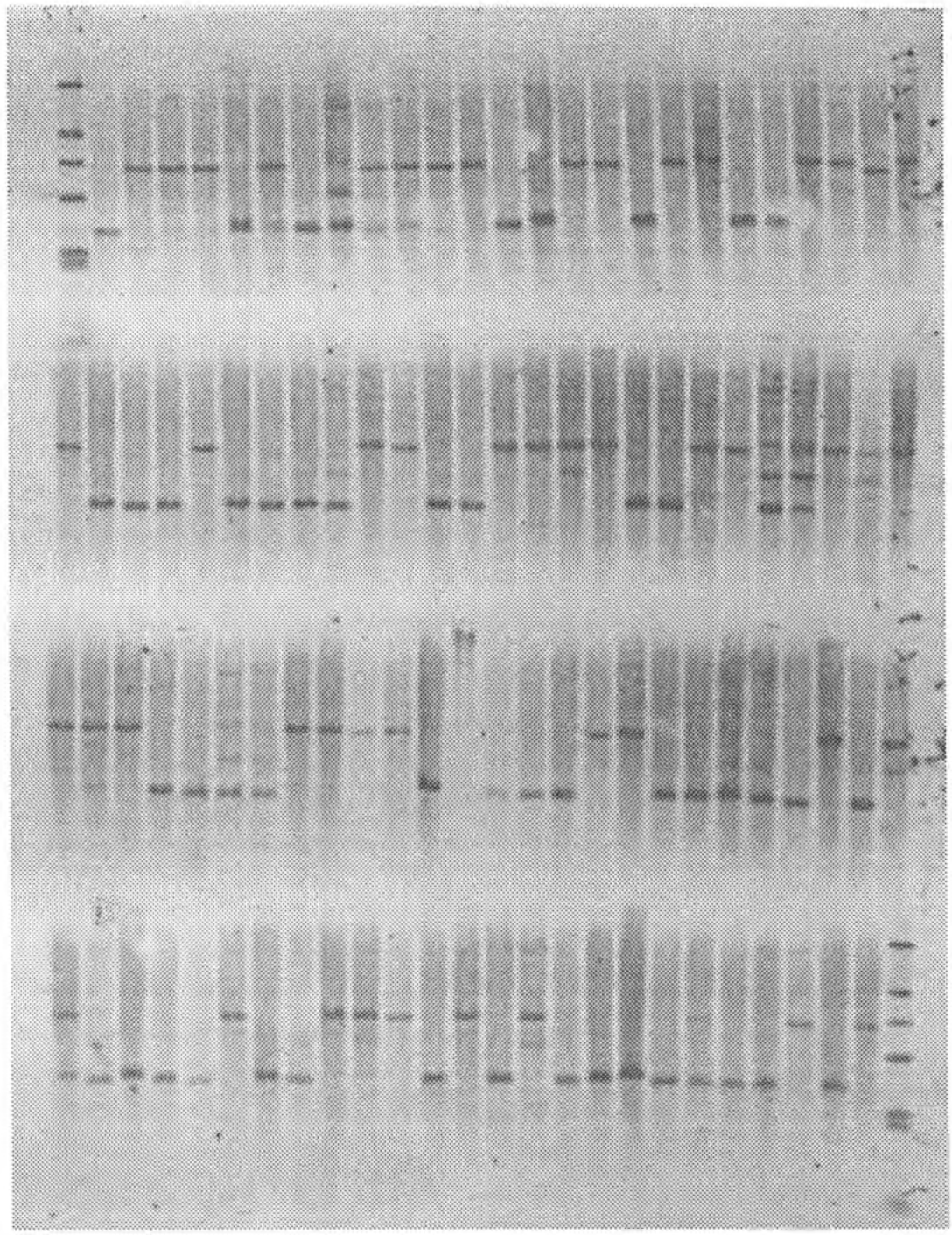

Figure. 4. 2. Segregation of 100 RILs of maize (Hi34 x TZi17) for RFLP marker *npi238*.

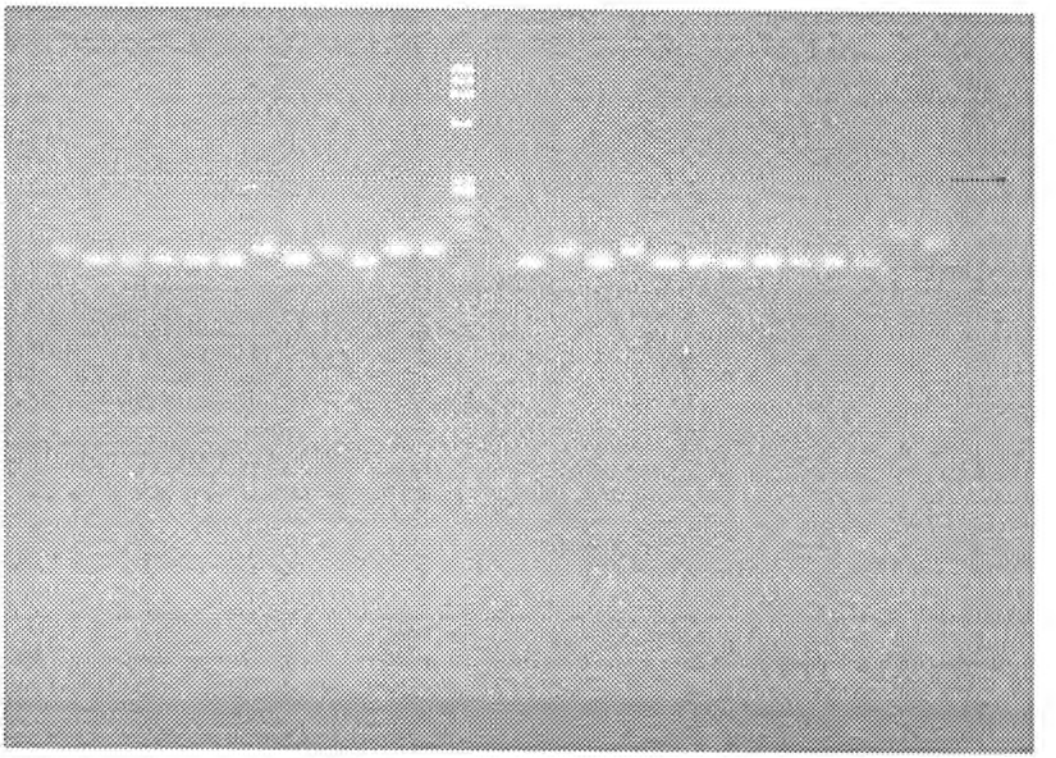

Figure 4.3. Segregation of SSR markers *phi022* in the RILs of maize (Hi34 x TZi17).

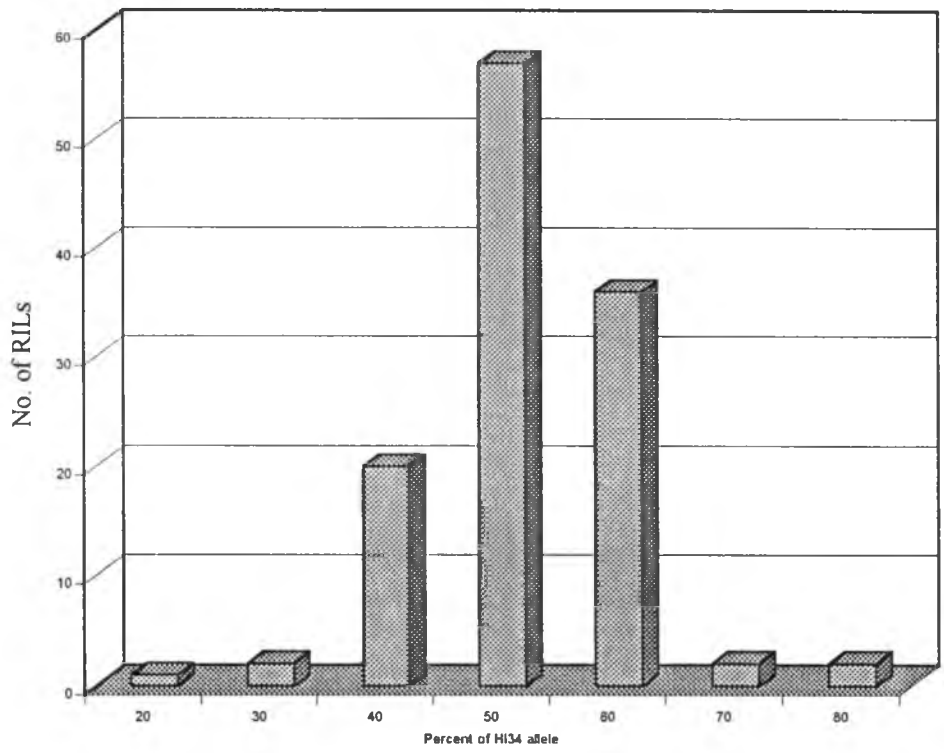

Figure 4.4. Distribution of percent of RFLP and SSR markers derived from inbred Hi34 among 110 RILs of maize (Hi34xTZi17).

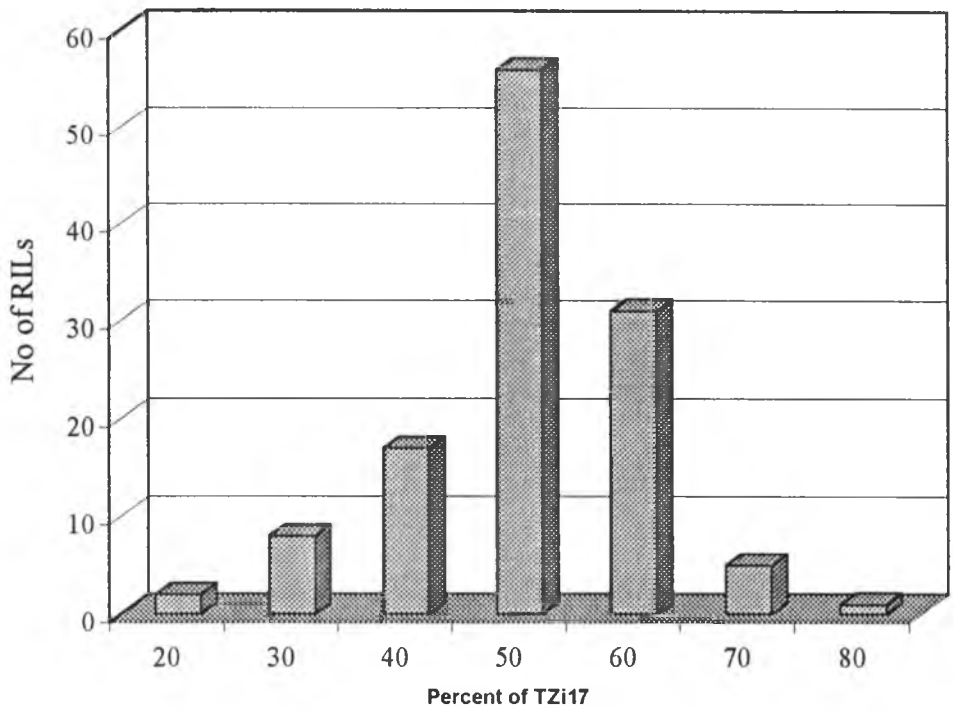

Figure 4.5. Distribution of percent TZi17 alleles for RFLP and SSR markers among 100 RILs of maize (Hi34 x TZi17)

markers from the MAPMAKER/EXP 3.0 program showed no major disagreement with the orders in the existing UMC RFLP map. Twelve RFLP markers and one SSR marker were excluded in map construction mainly because these markers showed segregation distortion and could not be assigned to the primary linkage groups. The constructed map in this study had 107 markers with a total length of 1984 cM and an average interval length of 18.5 cM.

4.3.4. Mapping QTLs for Resistance to Maize Streak Virus

Single-factor analysis of variance was used to determine the significance of association between RFLP marker genotype classes and MSV resistance in both 1992 and 1993 trials. The marker with the highest F-value was *umc167* in both trials (F = 10.9 in 1992 and F = 13.0 in 1993). The combined means of the RILs were analyzed in the single-factor analysis due to the consistent association between markers and MSV resistance in both trials. A total of nine markers showed significant correlation with QTLs for resistance to MSV by analyzing the combined data of both trials (Table 4.2). Seven of them were located on chromosome 1 near the marker *umc167* and this marker had the highest F values (F=17.44, P<0.0001), indicating a major QTL for resistance to MSV was linked to marker *umc167* on chromosome 1.

The putative QTL for resistance to MSV was further confirmed by using interval mapping. The peak LOD scores from interval mapping were 3.1 in 1992 and 5.8 in 1993. These appear to satisfy the LOD threshold values 3.5 as calculated from the 1000

Table 4. 2: Loci significantly associated with MSV resistance from single-factor analysis of variance

Locus	Chromosome bin[a]	F (1, n-2)	probability (F)
umc157	1.02	5.468	0.021
asg75	1.03	7.968	0.006
asg30b	1.04	11.841	0.001
umc167	1.05	17.439	0.000
umc166	1.--[b]	4.085	0.046
csu61	1.06	4.718	0.032
asg62	1.07	5.188	0.025
bnl6.25	2.01	4.260	0.042
bnl7.49	10.07	5.205	0.025

a Bin locations are designed by an X.Y code, where X is the linkage group containing the bin and Y is the location of the bin within the linkage group (Gardiner *et al.*, 1993)

b Not Clear

permutation tests (Churchill and Doerge, 1994) and using the combined average of the RILs from the two trials. Interval mapping placed the putative major QTL for MSV resistance approximately 6 cM from marker *umc167* and 14cM from marker *asg30*. This QTL accounted for 29.6% of the phenotypic variation with a peak LOD score of 6.0 (Model 3 in Figure 4.6). The resistant allele at this locus is present in the resistant parent and must trace back to the RppSR composite used in transferring MSV resistance to TZi17.

Composite interval mapping was also used in the mapping of the major QTLs for MSV resistance (Model 6 in Figure 4.6). A total of nine markers, including *umc167, umc157, umc102, csu39, bnl4.06, csu146, umc149, phi115*, and *umc113*, were excluded as background markers in the composite interval mapping based on the FB stepwise regression analysis. The peak positions from the interval mapping and from composite interval mapping were almost the same in LOD scores, confirming a major QTL located on chromosome 1. The LOD score distribution from composite interval mapping was narrower, however, than that from interval mapping method. Composite interval mapping method may have been more precise because it eliminates background markers and in this case alleviates the effect of the markers near *umc167*.

Another QTL for resistance to MSV was mapped on chromosome 9 by the composite interval mapping method with a LOD score of 3.0 (Figure 4.7). The map position of this QTL is between marker *umc113* and *bnl8.17* on Chromosome 9, and this QTL explained 5.9% of the phenotypic variation in MSV disease resistance. However,

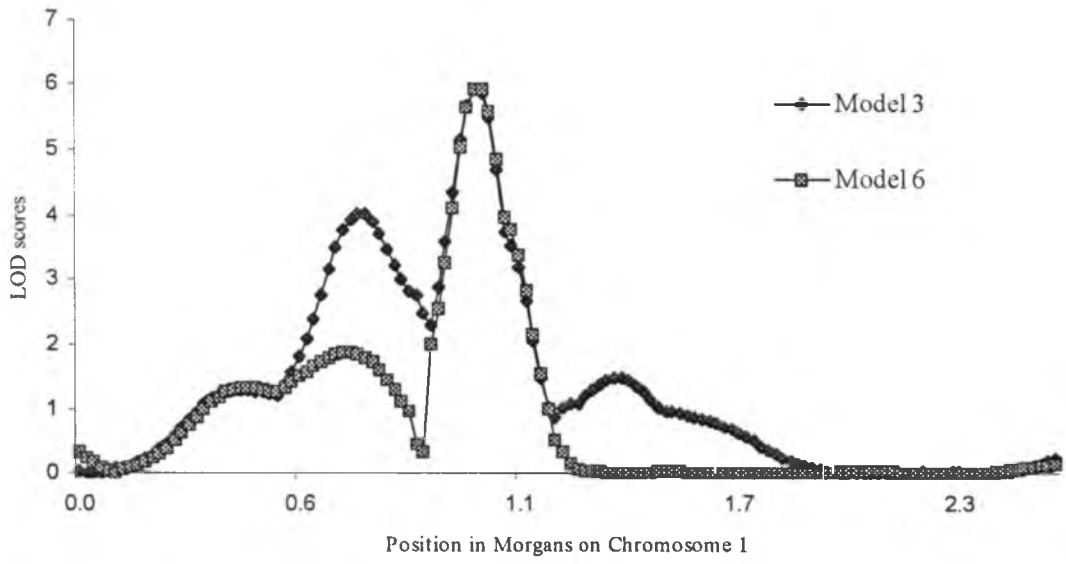

Figure 4.6. LOD scores of the region around the major QTL for MSV resistance on chromosome 1. Model 3 is for interval mapping and Model 6 is for composite interval mapping.

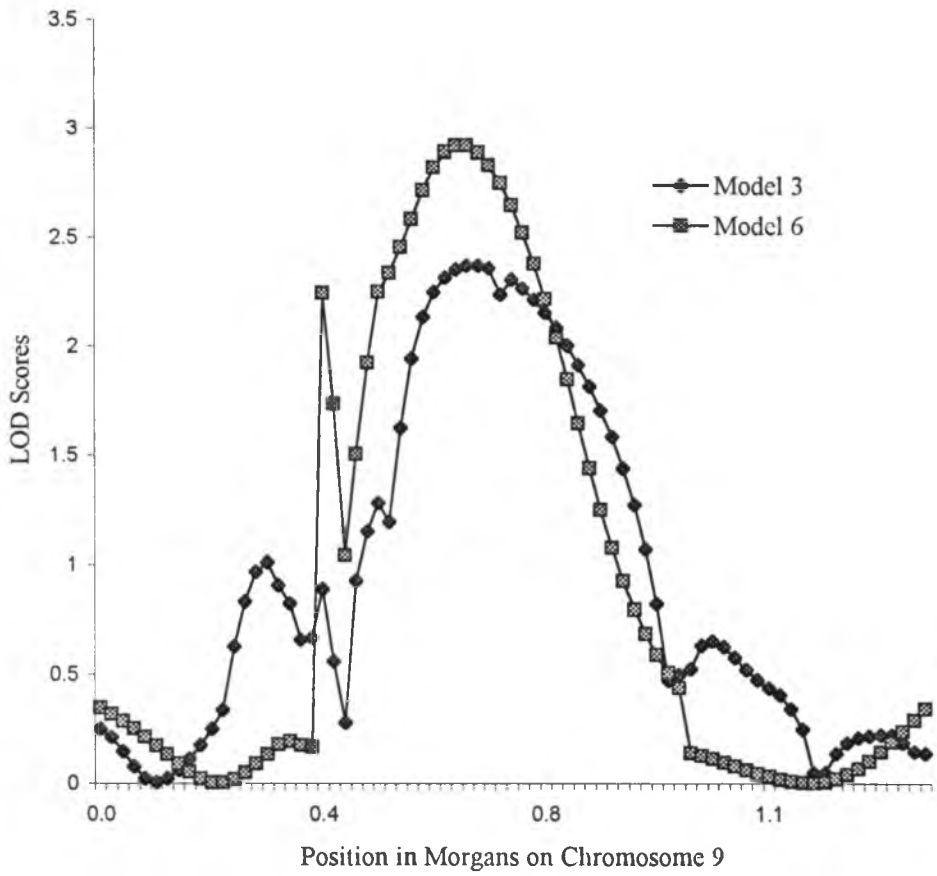

Figure 4.7. LOD scores of the region around the minor QTL for MSV resistance on chromosome 9. Model 3 is for interval mapping and Model 6 is for composite interval mapping.

single factor analysis did not reveal a QTL in this region, and the peak LOD score from interval mapping and composite interval mapping didn't exceed the threshold value. Comparing the LOD scores of this QTL and the major QTL on chromosome 1, it was concluded to be a minor or modifying QTL.

No significant ($P < 0.01$) digenic epistatic effects were found among QTLs for MSV resistance in the RILs. Similarly, no significant ($P < 0.001$) interaction between the detected QTL and loci in the rest of the genome.

4.4. Discussion

Two QTLs conferring resistance to MSV were identified in this study. The major QTL was located on chromosome 1 between RFLP marker *asg30* and *umc167*, 6 cM from marker *umc167* and 14 cM from marker *asg30*. Minor QTL was located on chromosome 9 in the region between marker *umc113* and *bnl8.17*. Kyetere *et al.* (1995) mapped a single major gene for resistance to MSV based on a Hawaii-bred set of RILs from TZi4 x Hi34 (Kyetere *et al.*, 1995). Although the sources of resistance to MSV were different, the major QTL for resistance to MSV identified in this study is almost in the same position as reported by Kyetere *et al.* (1995). Thus we can confirm that a major QTL conferring MSV resistance, designated *msv1* by Kyetere *et al.*(1995), is located on the short arm of chromosome 1. This major QTL was widely distributed in the MSV resistant germplasms. In the present study, a modifying minor QTL for resistance to MSV was shown to be located on Chromosome 9.

McMullen and Simcox (1995) reported that the majority of disease and insect resistance genes or QTLs appear to occur in clusters. The major QTL identified in this study was closely linked to the QTLs reported for resistance to fungal or bacterial disease such as *hm1,* conferring Curvularia leaf spot resistance, a QTL for Fusarium stalk rot resistance, and *sw1,* for Stewart's bacterial wilt resistance (Ming *et al.*, 1998). Also the minor QTL for resistance to MSV identified on chromosome 9 in the present study was closely linked to the minor QTL for resistance to Stewart's bacterial wilt and to *hm2* for Curvularia leaf spot resistance. This also supported the hypothesis that the clusters of resistant genes on chromosomes 1 and 9 derived from the same ancestral locus, as proposed by Helentjaris (1995).

CHAPTER FIVE

MOLECULAR MAPPING OF QTLS CONFERRING RESISTANCE TO CORN HEAD SMUT (*Sphacelotheca reiliana* (Kühn) Clint)

Abstract

A gene affecting resistance to maize head smut (*Sphacelotheca reiliana* (Kühn) Clint) was mapped using field-scored data of disease under natural infestation and molecular marker data. The mapping populations included the susceptible parent Hi34, the resistant parent TZi17, and 92 recombinant inbred lines (RILs) derived through single seed descent. Based on the analysis of 116 restriction fragment length polymorphisms (RFLP) and 4 simple sequence repeats (SSR), markers on the short arm of Chromosome 1 showed the largest effects indicating the existence of one major gene conferring resistance to *Sphacelotheca reiliana* (Kühn) Clint in this region. This gene, designated as *spr1*, was further mapped in the region between RFLP marker umc157 and asg30 on the short arm of chromosome 1, and it accounted for 10.6% of the phenotypic variation. Epistatic interactions also contributed an important role in the resistance to *S. reiliana*, especially involving loci on the long arm of chromosome 7.

5.1. Introduction

Head smut (also known as tassel smut) of corn is caused by the fungus *Sphacelotheca reiliana* (Kühn) Clint (syn. = *Ustilago reiliana, Sorosporium reilianum* (Kühn) McAlp). The disease was first observed in Kansas during the 1890 growing season

(Norton, 1895). Since then it has been observed in North America, Mexico, Australia, New Zealand, S. Africa and Europe. Head smut is a soil-borne, systemic disease (Smith and White, 1988). The incidence of the disease is dependent on the moisture, temperature, and pH of the soil at the time of planting (Whythe and Gevers, 1988). Two cultivars of the pathogen have been identified. One infects both corn and sorghum with four physiologic races. The other cultivar infects only corn with no physiologic races identified. The production of smut sori or exhibition of phylloidy on the reproductive parts of the plant can result in loss of yield. Losses due to the disease are generally minor, but individual fields may lose 30 to 80% of yield when epidemics occur. Infected plants are usually stunted to some degree.

Chemical control such as in-furrow soil treatment with fungicides (Stienstra *et al.*, 1985) or field management such as crop rotation and irrigation management (Mack *et al.*, 1984) reduces epidemics of the disease. However, genetic resistance is an ecologically and economically sound approach to the disease control. Differences in resistance to *S. reiliana* have been observed among many corn hybrids and inbreds. It has been proposed that there are both additive and dominant gene effects for the expression of resistance to *S. reiliana* (Whythe and Gevers, 1988). However, no definitive chromosomal assignment has been made for head smut resistance genes.

Major quantitative trait loci (QTLs) with large genetic effects provide the basis for rapid genetic gain with quantitative traits like disease and insect resistance (Moon *et al.*, 1999). Several disease resistance genes with major effects have been mapped in the corn

genome by molecular marker linkage analysis (Ming *et al.*, 1997, 1999; Lu *et al.*, 1999). These resistant genes include maize dwarf mosaic virus (Simcox *et al.*, 1994), maize mosaic virus (Ming *et al.*, 1997), maize streak virus (Kytere *et al.*, 1995; Lu *et al.*, 1999), Curvularia leaf spot (*Cochliobolus carbonum* Nelson) (Coe *et al.*, 1988), southern corn leaf blight (*Bipolaris maydis* (Nisik.) Shoem) (Zaitlin *et al.*, 1993) and Stewart's wilt (*Erwinia stewartii* Smith) (Ming *et al.*, 1999).

A set of recombinant inbred lines (RILs) derived from the cross between a highly resistant inbred, TZi17, and a susceptible inbred, Hi34, was used to construct a marker map (Lu *et al.*, 1999) and to evaluate the resistance to *S. reiliana.* The purpose of this study was to map and to characterize the head smut resistance gene(s) in these materials.

5.2. Materials and Methods

5.2.1. Disease Nursery

Field evaluation for resistance to head smut was carried out in the Greytown, South Africa by Dr. David Nowell, an expert on head smut of Pioneer Co., during the 1993 crop cycle (Moon, 1995). The experimental design was an 11 x 11 double lattice design with two replications. The total 121 entries include the 100 RILs, the F_1, and ten sub-lines each from the two parents. Each plot was planted in single row 5 m long and with 75 cm spacing between rows.

The head smut screening was under natural infection due to the epibiotics of *S. reiliana* in the field environment, especially in the soil. The number of plants with smut on the tassels was counted for each plot. The percentage of smutted plants of each plot was transformed to a 1-9 scale. Rating of 1 through 8 were applied when the number of smutted plants were of 0, 5, 10, 20, 30, 40, 50, 60% of the total numbers of the plants in the plot, with rating 9 in excess of 60%.

5.2.2. Statistical Analyses

QTLs for head smut resistance were identified by using QTL Cartographer 1.12f (Basten *et al.*, 1997) for composite interval mapping (Zeng,1994). Composite interval mapping is a refinement of interval mapping (Lander *et al.*, 1987), in which the test of each interval is unaffected by QTLs in other regions of the genome. This is done by including marker loci, identified by stepwise regression that explained the most variation for the phenotypic variation, as cofactors in the interval mapping. We used Model 6 of the Zmapqtl procedure of QTL Cartographer, scanning the genome every 2 cM. We also calculated genome-wise threshold ($\alpha = 0.05$) based on 1000 permutation test (Churchill and Doerge, 1994; Basten *et al.*, 1997).

To estimate the proportion of phenotypic variance accounted for by the detected QTL, we conducted single factor analysis of variance (SFA) with the SAS GLM procedure. The R^2 value (coefficient of determination) from this analysis was accepted as the percent phenotypic variance explained by the locus. We also evaluated multiple-locus

models, including the significant digenic epistatic interactions, with SAS GLM procedure. Digenic epistatic interactions between all pairs of loci were evaluated by maximum likelihood methods together with Monte Carlo simulations, as incorporated into the program Epistat (Chase *et al.*, 1997).

5.3. Results

5.3.1. Phenotypic Data Analysis

The two parental lines differed significantly ($P<0.01$) for resistance to *S. reiliana.* The ten sub-lines each of resistant parent TZi17 and susceptible parent Hi34 averaged 2.4 (range from 1 to 4.5) and 5.8 (range from 1.4 to 8.4). The F_1 hybrid averaged 5.5 and ranged from 4.5 to 6.5, indicating that susceptibility to *S. reiliana* was partially or incompletely dominant.

The average scores for RILs ranged from 1 to 9 for resistance to *S. reiliana.* Variation among the RILs was highly significant ($P<0.001$) and the distribution of resistance deviated significantly from normality (Figure 5.1). The observed bimodal distribution among RILs for resistance approximated closely that expected for a single major QTL by both the normal distribution method ($X^2 = 15.1$, $P<0.01$) and maximum likelihood estimation ($LR = 3.8$, $P<0.05$) (Moon *et al.*, 1999).

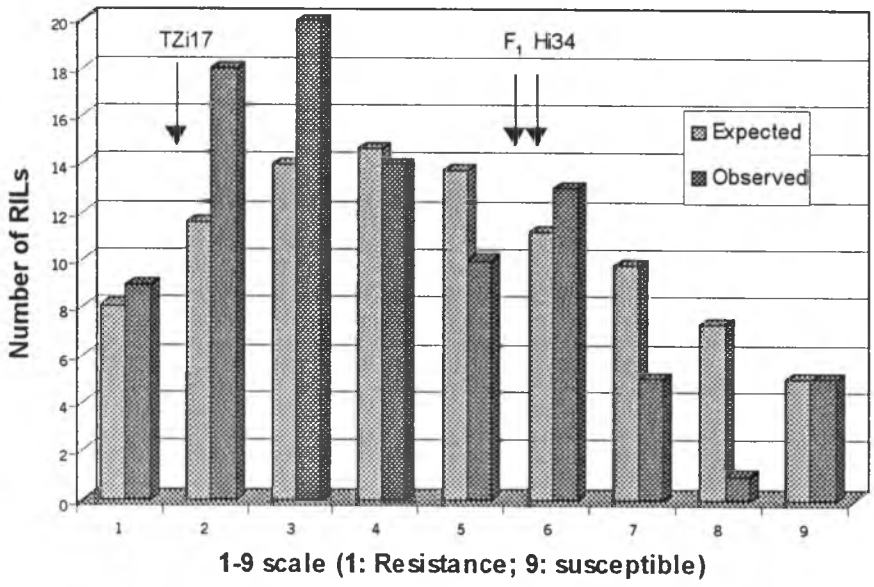

Figure 5.1: Mean disease rating of 92 RILs derived from Hi34 x TZi17 for resistance to head smut with expected values based on model of monogenic segregation.

5.3.2. Mapping S. reiliana Resistance Gene

The results of the molecular marker analysis and linkage map for the RILs of population Hi34 x TZi17, based on 116 RFLP and 4 SSR marker loci, have been presented by Lu *et al.* (1999). The constructed linkage map had a total length of 2060 cm and an average spacing of 18.7 cm between markers.

Identification of marker loci linked to *S. reiliana* resistance was conducted using single factor analysis of variance. Marker loci on chromosomes 1, 2, 9, 10 carried genes influencing resistance (Table 5.1). The most influential region for resistance to *S. reiliana* was located in the proximal portion of chromosome 1, where RFLP marker asg30 showed the highest F value (F=10.17). Two linked markers, umc167 and asg75, were also associated highly with resistance to *S. reiliana*.

The data suggest a major QTL for resistance to *S. reiliana* on the short arm of chromosome 1 closely linked to marker *asg30*. This resistance allele originated from resistance parent TZi17. For the 27 RILs showing high resistance (disease score less than 2), 22 had *asg30* from the resistant parent TZi17, while 5 lines showed *asg30* loci from the susceptible parent Hi34. Sixteen of the 22 highly susceptible RILs (disease score higher than 6) had *asg30* loci from the susceptible parent Hi34, and 6 lines showed the *asg30* locus from the resistant parent TZi17.

A LOD score of 4.2 was set as the genome-wise threshold value at $P<0.05$ for identifying putative QTL based on the 1000 permutation test from QTL Cartographer. A

Table 5.1: Loci significantly associated with resistance to corn head smut from single-factor analysis of variance

Locus	Chromosome bin[a]	F (1, n-2)	probability (F)
asg75	1.03	5.327	0.023
asg30	1.04	10.168	0.002
umc167	1.05	4.591	0.035
umc50	3.04	4.349	0.040
phi022	9.03	5.115	0.026
csu25b	10.00	7.408	0.008

a. Chromosome bin locations are designed by an X.Y code, where X is the linkage group containing the bin and Y is the location of the bin within the linkage group (Gardiner *et al.*, 1993).

scan of all ten chromosomes using composite interval mapping by QTL Cartographer revealed only one peak. This was on the short arm of chromosome 1 close to the marker *asg30*, with LOD score of 5.3 that accounted for 10.6% of phenotypic variation for head smut. The map position was in bin1.04, about 4 cM from marker *asg30* and 16 cM from *umc167*. The data all support the contention that a single major gene, here designated as *spr1*, confers resistance to *S. reiliana* (Figure 5.2).

Seven pairwise epistatic interactions ($P<0.01$) for resistance to *S. reiliana* were detected. Each of the three most significant pairwise epistatic interactions ($P<0.001$) involved one marker in the long arm of chromosome 7. The mixture model, including the resistance allele *spr1* and all three interactions with markers of chromosome 7, could explain up to 60% phenotypic variation (SAS GLM).

5.4. Discussion

Recombinant inbred lines (RILs) are powerful genetic tools and are particularly useful in plants because large numbers of RILs can be prepared and stored as seed. RILS are highly homozygous with a mixture of genes from the two parents as a result of chromosome segregation and recombination. Because of the genetic constancy of RILs, different experiments for different genetic analyses can be carried out in different environments and/or at different times. The data reviewed here come from a single evaluation, in S. Africa, so RILs would wisely be repeated for a second data set.

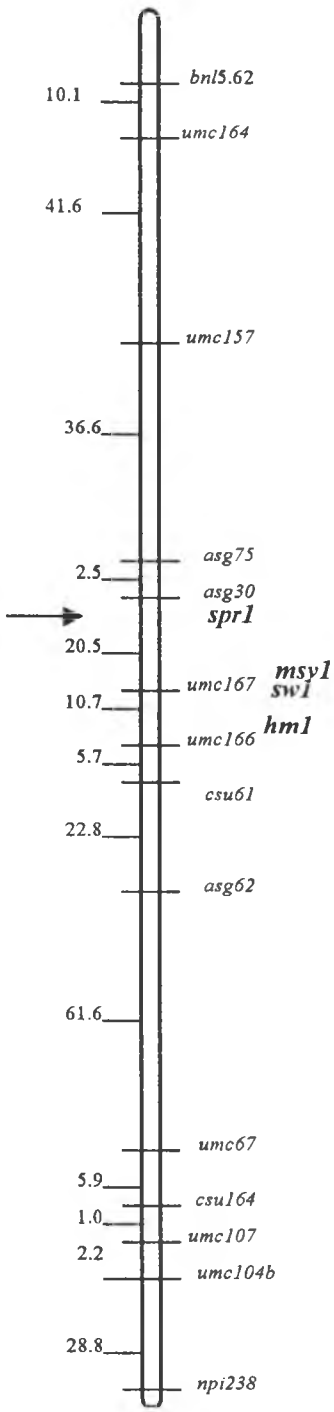

Figure 5.2. Genetic map of the region around the *spr1* locus (*arrow*) on chromosome 1. Genetic distance are shown in CentiMorgans to the *left*. The map was generated from the analysis of 92 RILs derived from Hi34 X Tzi17. The relative map positions of *msv1*, *sw1*, and *hm1* are shown to the *right*.

The *S. reiliana* resistance gene *spr1* was located on the short arm of chromosome 1 between RFLP marker *asg30* and *umc167*. This map position placed *spr1* close to several other genes that confer resistance to viral, fungal, and bacterial diseases. It is in proximity to *msv1*, conferring resistance to maize streak virus, and can be traced back from the same origin parents TZi17 in the same RILs (Lu *et al.*, 1999). It is also close to *hm1*, conferring resistance to Carbonum leaf spot (Coe *et al.*, 1988), and *sw1*, for resistance to Stewart's bacterial wilt (*Erwinia stewartii* Smith) (Ming *et al.*, 1999). This resistance gene cluster might be a classic example in maize genome for future gene tagging or for the study of evolution conservation. It also suggests that there may be some other resistance genes located in this region (McMullen and Simcox, 1995).

Genetic map comparisons make it clear that gene composition and order are commonly conserved among plants, especially among the cereals. There is a clear alignment of the maize dwarfing loci, *br1, an1* and *py1*, with QTLs for plant height in sorghum (Pereira and Lee, 1995). The major gene synteny between sorghum and maize for seed weight, a key component of domestication of crop plants, indicated that maize chromosome 1 was collinear with Sorghum linkage group C (Paterson *et al.*, 1998). This may imply the existence of a disease resistance gene cluster in sorghum in linkage group C, especially for head smut resistance resulted from at least the same cultivars of pathogen as corn.

The mapping of QTLs conditioning head smut resistance in maize should assist in the management of maize germplasm for resistance to head smut. Furthermore it may lead to a degree of genetic control able to reduce the utilization of chemicals. The gene, *spr1*, identified here should be transferable by conventional selection. DNA marker prescreening might also be used to reduce the tedious field evaluations of tassel disease, and would be particularly attractive for use where field release of the pathogen is forbidden. Also the epistatic interactions for resistance to *Sphacelotheca reiliana* (Kuhn) Clint involving the marker loci on chromosome 7 might be accomplished by DNA markers, although it was impossible to handle by traditional manipulation.

CHAPTER SIX

MAPPING OF QANTITATIVE TRAIT LOCI CONFERRING GENERAL RESISTANCE TO COMMON RUST IN MAIZE

Abstract

Common rust, caused by *Puccinia sorghi* Schw., is a serious disease of maize (*Zea mays* L.) worldwide. Deployment of durable resistant varieties is desirable both economically and environmentally. Recombinant inbred lines (RILs) from a cross between a resistant inbred Hi34 and a susceptible inbred TZi17 were evaluated for common rust resistance for three seasons at two locations under natural infestation. Based on molecular marker analyses, regions on chromosome 6 and 9 were consistently associated with general or race-nonspecific resistance to common rust by composite interval mapping (CIM), accounting for about 16.1% and 12.9% of disease variations. These DNA markers may be useful in characterizing general resistance genes and in breeding durable resistant maize varieties that accumulate both general and race-specific resistance genes.

6.1. Introduction

Fungal rusts of the genus *Puccinia* are among the most devastating pathogens in agriculture worldwide (Smith and White, 1988). Common rust of maize is caused by the fungus *Puccinia sorghi* Schw., can cause losses in both yield and quality under favorable

conditions (Pataky *et al.*, 1988). Deployment of genetic resistance is the most economical and effective way of controlling the disease (Brewbaker, 1983). Resistance to common rust is clearly of two types based on pathogen reproduction: race-specific and general or non race-specific (Hooker, 1969; Kim and Brewbaker, 1977).

Race-specific resistance of maize to common rust is characterized by hypersensitive response to infection, evident even at the seedling stage. Inheritance is commonly monogenic by Rp loci, with resistance dominant or partially dominant. Rp genes in maize have been located in five genomic areas (Hulbert, 1997). Race-specific resistance genes interact with the corresponding genes for avirulence in fungal pathogens in a gene-for-gene manner (Abedon and Tracy, 1998). Race-specific resistant genes are often tightly clustered in the genome (Hulbert, 1997). The Rp loci that specify resistance to common rust disease have been particularly well characterized. Most of the 25 Rp genes characterized by Hooker (1969) mapped to the Rp1 area on the short arm of chromosome 10, which also includes genes designated Rp5 and Rp6 that span two or more cM. Kim and Brewbaker (1987) identified a single recessive gene, designated as rp-677 and closely linked to Rp-d that controlled resistance to *P. sorghi* in sweet corn inbred IL677a. Other Rp loci were Rp4 on chromosome 4 and Rp3 on chromosome 3 (Hagan and Hooker, 1965; Hooker, 1969; Sanz-Alferez, 1995). Recently, two more loci were identified as Rp7 and Rp8, which segregate independently of Rp1, Rp3, and Rp4 (Hulbert, 1995; Delaney *et al.*, 1998). The map position of Rp7 is not clear. Rp8 locus

was mapped on the short arm of chromosome 6 with unique inheritance pattern (Delaney *et al*, 1998).

General resistance to common rust is pathogen race-nonspecific. It limits disease development by reducing pustule number, size, and sporulation per pustule (Pataky, 1986), and is often referred to as mature plant resistance. General resistance to common rust is quantitatively inherited and has high heritability, ranging from 60 to 90% (Hooker, 1969; Kim and Brewbaker, 1977; Randle *et al.*, 1984). Kim and Brewbaker (1977) estimated that as few as two gene loci conditioned general resistance. There have been few reports on the effectiveness of selection for general resistance (Randle *et al.*, 1984; Gingera *et al.*, 1994; Abedon and Tracy, 1998).

General or mature plant resistance should be used in breeding (Hooker, 1969), because race-specific resistance may break down under severe epiphytotics (Brewbaker, 1983; Groth *et al.*, 1992). All race-specific loci have become ineffective in Hawaii, although loci such as Rp1-D (widely used on the mainland) once provided resistance (Kim and Brewbaker, 1977). General resistance is effective against all tested biotypes of *P. sorghi* (Smith and White, 1988).

The development of molecular marker techniques makes it possible to investigate the inheritance of general resistance and to locate and manipulate individual quantitative trait loci (QTLs) associated with the disease (Tanksley, 1993; Wang *et al.*, 1994; Ming *et al.*, 1997; and Nelson *et al.*, 1997). Recently RFLP maps of maize chromosome were constructed in a recombinant inbred line (RILs) population segregating for rust resistance

(Lu *et al.*, 1999). The objective of our study was to identify chromosome regions carrying common rust resistance genes.

6.2. Materials and Methods

6.2.1 Field Trials

Three field experiments for the evaluation of common rust resistance were conducted in Mexico at the Poza Rica experiment station of CIMMYT by Dr.Ganesan Srinivasan during 1994 (Exp.1), at Waimanalo Research Station of University of Hawaii by Dr. Hyeon Gui Moon during 1994 (Exp.2) and 1997 (Exp. 3). The 120 entries included 100 RILs and ten sub-lines from each of the two parents. They were planted in a randomized complete block design with two replications in all three trials. Rows were 5 m long and 75 cm between rows. All trials were under natural infection with the epibiotics of the pathogen in the tropical environment. Plants were scored 3 weeks after mid-silking on a 1-9 scale for percentage of leaf area covered with rust, excluding the upper three leaves. Ratings of 1 through 8 were applied when rust comprised 1, 5, 10, 20, 30, 40, 50, and 60% of the leaf surface, with rating 9 in excess of 60%.

6.2.2. Data Analysis

The PROC GLM procedure in the Statistical Analysis System (SAS) was used to determine association between molecular markers and resistance to common rust in each

trial. QTL Cartographer version 1.12 was also used to identify putative quantitative trait loci conferring resistance to common rust based on composite interval mapping (Basten *et al.*, 1997; Zeng, 1993). The significant threshold for QTL detection was derived from 1000 permutation test using the QTL Cartographer version 1.12 (Churchill and Doerge, 1994; Basten *et al.*, 1997).

6.3. Results

6.3.1. Agronomic Trials

The two parental lines differed significantly ($P<0.01$) for the response to common rust epibiotics in all three trials. The average disease score of Hi34 over the three trials of field evaluation was 3.9, while that of TZi17 was 6.8. Several RILs were missing in each of the three trials. Continuous distribution of common rust resistance of the RILs in each field experiment (Table 6.1) indicated field resistance to be controlled by more than one gene. However the result from both normal distribution method (Moon *et al.*, 1999) and maximum likelihood estimation indicated the presence of major gene(s) responsible for resistance to P. *sorghi* among the RILs (Appendix 3). Transgressive segregation was observed for the response to common rust in the RILs. The correlation coefficients among the RILs from all three trials were highly significant ($P<0.01$).

Table 6.1. Distribution of Hi34 x TZi17 RILs for common rust resistance in three trials at Poza Rica, Mexico and at Waimanalo, Hawaii.

		Numbers of RILs with disease scores								
Trials	Locations	1	2	3	4	5	6	7	8	9
Exp1	Mexico	0	6	16	12	5	23	21	7	1
Exp2	Mexico	0	0	2	2	11	34	26	16	1
Exp3	Hawaii	0	1	14	15	23	18	10	3	0

6.3.2. Mapping Genes for General Resistance to Common Rust

The results of the molecular marker analysis, including the linkage map for the RIL of population Hi34 x TZi17, based on 116 RFLP and 4 SSR marker loci, have been presented in our companion paper (Lu *et al.*, 1999). The constructed linkage map had a total length of 2060 cm and an average spacing of 18.7 cm between markers.

Single factor analysis by SAS PROC GLM indicated several chromosomal regions that influenced common rust resistance in different experiments (Table 6.2). The most consistent regions were on chromosomal 6 and 9. In all three trials, association of resistance with *umc59* (bin 6.02) was significant. Two DNA markers on chromosome 6 (*umc113* and *phi022*) were significantly correlated with disease resistance in two of the three trials. These results suggest both chromosome 6 and chromosome 9 have important genes conferring common rust resistance across the three environments.

A LOD score of 3.5 was set as the threshold for detecting a putative QTL based on the 1000 permutation test from QTL Cartographer 1.12. This critical value is equivalent to a significance level $\alpha = 0.10$ in the analysis of one trait in the present study.

Composite interval mapping (Zeng, 1993, 1994) revealed two QTLs conferring general resistance to common rust consistently across the three experiments. They were also located on chromosomes 6 and 9. The QTL on chromosome 6 (Figure 6.1) was located on the short arm again near the marker *umc59*. The LOD score in this region was 5.0 for exp1. The proportion of the phenotypic variation that explained by this QTL was

Table 6.2: Loci significantly associated with common rust resistance from single-factor regression analysis in 100 RILs (Hi34 x TZi17) tested in three trials at two locations†

		Exp1		Exp2		Exp3	
Locus	Bin‡	F(1,n-2)	P	F(1,n-2)	P	F(1,n-2)	P
bnl5.62	1.01	0.9	0.356	**5.4**	**0.023**	0.7	0.399
umc164	1.01	1.7	0.204	**4.5**	**0.036**	2.2	0.140
asg75	1.04	0.1	0.756	0	0.962	**5.3**	**0.023**
umc167	1.05	3.5	0.065	0.4	0.516	**4.5**	**0.036**
umc199	3.09	3.4	0.067	0.7	0.417	**4.4**	**0.038**
umc14	4.06	1.2	0.474	0.6	0.635	**6.5**	**0.012**
umc133	4.08	**6.9**	**0.010**	0.9	0.345	0.9	0.345
bnl6.25	5.01	0.9	0.351	1.3	0.252	**5.1**	**0.026**
umc59	6.02	**9.7**	**0.002**	**4.4**	**0.039**	**8.8**	**0.004**
umc38	6.06	**7.1**	**0.009**	1.1	0.278	2.2	0.144
umc113	9.01	**5.5**	**0.021**	**4.3**	**0.040**	0	0.837
phi022	9.03	**4.7**	**0.032**	2.4	0.122	**4.7**	**0.033**
npi285	10.02	**4.9**	**0.028**	2.0	0.160	2.1	0.152
umc130	10.03	3.2	0.075	3.2	0.077	**4.7**	**0.032**
csu46	10.04	3.8	0.053	**6.1**	**0.015**	2.1	0.154
npi232	10.05	2.7	0.107	**4.5**	**0.036**	1.0	0.052

† Significant correlations are in the bold format

‡ Bin locations are designed by an X.Y code, where X is the linkage group containing the bin and Y is the location of the bin within the linkage group (Gardiner *et al.*, 1993).

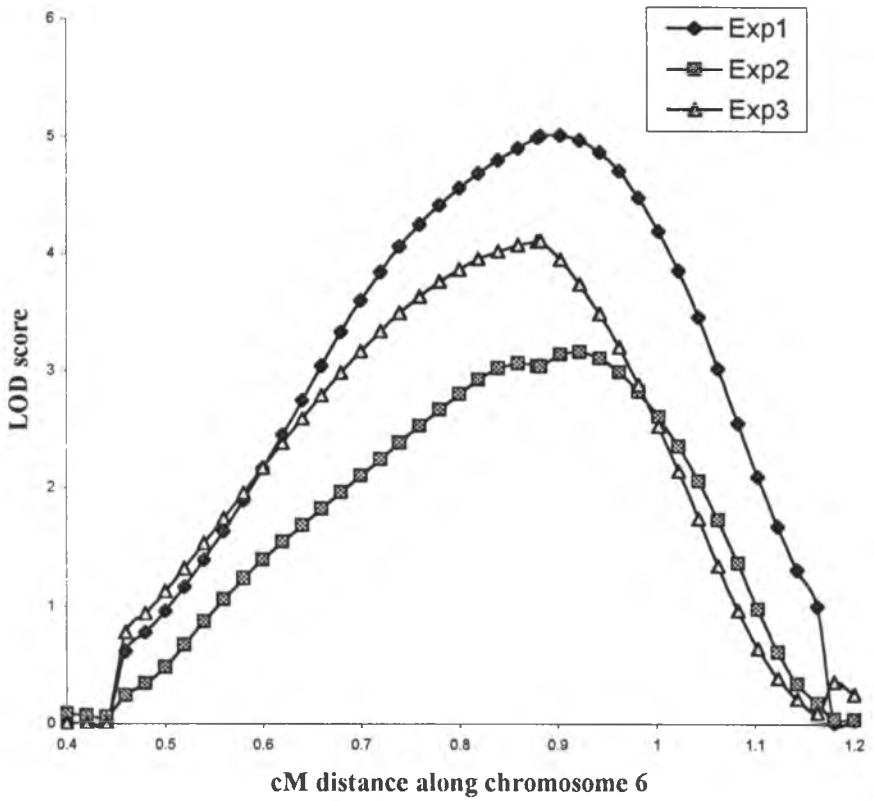

Figure 6.1. Quantitative trait loci conditioning general resistance to common rust on chromosome 6 as depicted by composite interval mapping in 100 RILs (Hi34 x Tzi17) tested in three trials at two locations.

12% in exp1. Both exp2 (LOD = 3.1, R^2= 0.073) and exp3 (LOD = 4.1, R^2= 0.091) supported this putative QTL, located about 2 cM distal to marker *umc59* on chromosome 6 based on the CIM procedure with combined means of all three experiments. The peak LOD score of this QTL was 7.3. This QTL was derived from parent Hi34 and accounted for about 16.1% of the phenotypic variation for rust resistance.

Another QTL identified for general resistance to common rust across all the three experiments was located on the short arm of chromosome 9 (Figure 6.2). This QTL also originated from parent Hi34. The LOD scores of this QTL were 2.8 for exp1, 3.5 for exp2, and 5.7 for exp3 based on the composite interval mapping procedure. The phenotypic variations that explained by this QTL was 8.6% in exp1, 6.1% in exp2, and 7.8% in exp3 (SAS GLM). Composite interval mapping of the combined mean values of the three trials resulted in 12.9% phenotypic variation explanation of this QTL, located about 1 cM distal to marker phi22.

Several other chromosome regions may also modify general resistance to common rust, as discovered in one trial only (Table 6.2). None of these regions had a LOD score exceeding or close to the significant threshold, indicating that these regions should not have major QTLs with significant effect on general resistance.

No significant digenic epistatic effects were found between these two QTLs for general resistance to P. *sorghi* in the three trials.

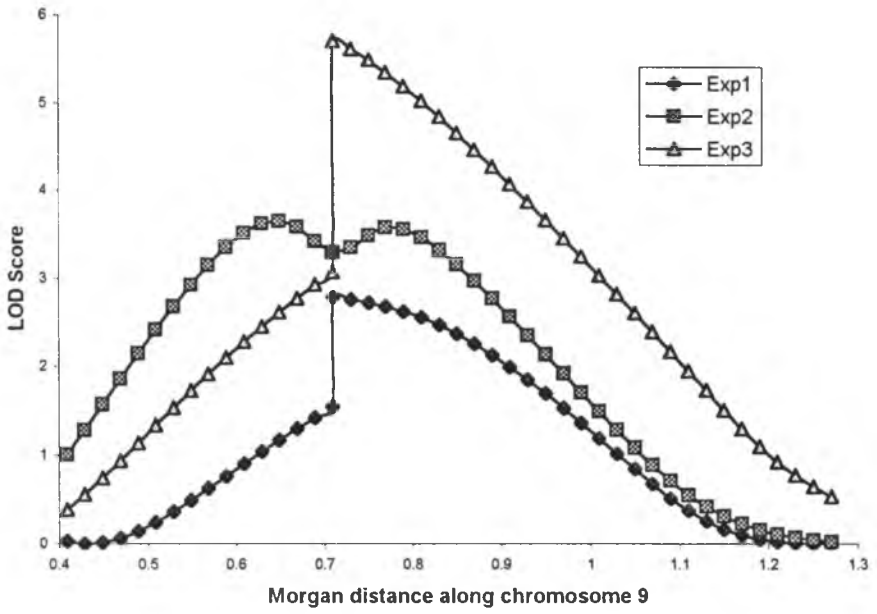

Figure 6. 2. Quantitative trait loci conditioning general resistance to common rust on chromosome 9 as depicted by composite interval mapping in 100 RILs (Hi34 x Tzi17) tested in three trials at two locations.

6.4. Discussion

Biotypes of common rust may differ in distinct field locations or growing seasons. Therefore RIL populations are ideal for the mapping of general resistance to common rust, since these populations can be repeatedly tested in different locations with various growing seasons. Additional RILs in the mapping population would also provide better resolution of the QTLs identified in this study.

None of the race-specific resistance genes was identified for general resistance to common rust in this study. The putative QTLs for the general resistance to common rust on chromosomes 6 and 9 in this study were designated as *qrp1* and *qrp2*, respectively. These two QTLs, *qrp1* and *qrp2*, appear to be closely linked to QTLs for general southern rust resistance (Ming, 1995), indicating the possibility of common resistance mechanism between the general resistant genes for both common rust and southern rust.

Comparative genetic mapping among cereal genomes of remarkably different complexity has demonstrated that homologous single copy sequence and (or) genes (cDNA) are collinear on the RFLP map of wheat, barley, oat, rye, maize sorghum, and rice (Gale and Devos, 1998). Paterson *et al.* (1995) identified convergent domestication of cereal crops by studying QTLs that affect seed mass, reduced disarticulation of the mature inflorescence, and daylength-insensitive flowering. They also identified chromosomal duplications within taxa such as the duplications on maize chromosomes 6, 9, and 10 that harbor QTLs affecting daylength-insensitive flowering. These three chromosomal regions correspond to *qrp1* on chromosome 6 and *qrp2* on chromosome 9

for general resistance to common rust, and to the Rp1 complex on chromosome 10 for race-specific resistance to common rust . This implies that chromosomal duplication may also account for the polygenic inheritance for common rust resistance in maize. Furthermore, it may also suggest a common genetic resistance mechanism between the race non-specific resistance and general resistance to common rust. In fact recently there was evidence of the derivation of a race-nonspecific resistance gene from race specific resistant genes. Hu *et al.* (1997) separated race non-specific resistant gene rp1-NC3 from the Rp1 complex in maize.

The combination of general and specific resistance is preferred to obtain durable resistance of cultivars in modern agriculture (Smith and White, 1988). Breakdown of elite varieties with specific resistant genes due to biotype evolution of the rust fungus is not uncommon. Genetic elucidation of general resistance and the genetic manipulation of QTLs by marker assisted selection (MAS) may be one of the solutions.

CHAPTER SEVEN

GENETICS OF RESISTANCE IN MAIZE TO THE CORN LEAF APHID

Abstract

The corn leaf aphid, *Rhopalosiphum maidis* (Fitch), is a major pest of hosts like maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), and sorghum (*Sorghum bicolor* (L.) Moench). The species is parthenogenetic and viviparous, and it serves as a vector for maize dwarf mosaic virus (MDMV) disease. Resistance was observed to characterize two inbreds, Hi34 and Hi38-71, in research conducted in Hawaii. Generation mean analysis was conducted on 6 generations of maize [P_1, Hi38-71 (resistant); P_2, G24 (Susceptible); F_1; F_2; BC_1 and BC_2] to determine the type of gene action involved in the resistance of Hi38-71. Resistance was shown to be monogenic and recessive in Hi38-71. Molecular markers were used to map the resistance loci to corn leaf aphid in a set of 100 recombinant inbred lines (RILs), derived from the cross between susceptible TZi17 and resistant Hi34. Analysis of 100 RILs by 120 marker loci suggested the presence of a major recessive gene for resistance to corn leaf aphid, tentatively designated *aph2*, on chromosome 2.

7.1. Introduction

The corn leaf aphid, *Rhopalosiphum maidis* (Fitch), is widely distributed over the world and has probably been closely associated with cereals and wild grasses for

thousands of years (Dicke, 1969). Its preferred hosts include maize (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench), and barley (*Hordeum vulgare* L.). Large corn leaf aphid populations have been associated with yield loss, particular under stress of high infestation and soil moisture (Rhodes and Luckmann, 1967; Dicke, 1969). Yield reduction up to 91.8% occurred in heavily infested maize under drought stress.

The corn leaf aphid reproduces by parthenogenesis and may develop large populations in a few weeks (Dicke, 1969). It undergoes four immature stages before maturing into either apterate (non-winged) or alate (winged) form. The alate form flies into the field to initiate the apterous colonies (all female), and the viviparous apterous form then builds up large populations. The development of the alate form is associated with population density and nutritional factors in the plant. High-density infestations and lowered nutritional content will provide the alate forms that disperse to establish new areas of infestation. The migration of the alate forms also vectors the virus disease, maize dwarf mosaic virus (MDMV) (Dicke and Sehgal, 1990).

Resistance to corn leaf aphids in maize was first reported by Gernert (1917), who showed F_1 hybrids of annual teosinte crossed with yellow dent maize to be resistant. Chang and Brewbaker (1974, 1976) reported a mongenic recessive resistance allele in the AA8sh2 populations. Bing *et al.* (1991) suggested that multiple genes are involved in corn leaf aphid resistance based on nine generation mean analyses derived from a susceptible parent (B96) and resistant parent (Mo17), and from diallel analyses of ten inbred lines showing various resistance to corn leaf aphids. Resistance to corn leaf aphids has been

reported also to be influenced or modified by many factors, including morphology, soil and climate conditions, and physiological factors. In Hawaii the evaluations of resistance are highly influenced by predators and parasites, that around in field since no insecticides are used.

One objective of the present study was to analyze six generations of maize resulting from the cross between aphid resistant Hi38-71 and susceptible genotype G24 using the six parameter genetic model (Hayman, 1958, 1962). The model allows for determination of genetic effects based on estimates from generation means, and differentiates between additive, dominance and interaction (epistatic) effects. A second objective was to map QTLs for resistance to corn leaf aphids based on the molecular marker analysis of 100 RILs derived from a cross between a susceptible parent, Hi34, and a resistant parent, TZi17.

7.2. Materials and Methods

7.2.1. Generation mean analysis

The experiment was designed as a randomized complete block with two replications. The experiment was planted at the Waimanalo Research Station of University of Hawaii. Field plots were planted in single 5-m rows on 9 June 1998, with 0.75-m spacing between rows. Each replicate consisted of 6 generations: the parents P_1 (Hi38-71 resistant) and P_2 (G24 susceptible), and the F_1, F_2, and backcrosses to both parents (BC_1

and BC_2). Hi38-71 is a subline derived from supersweet corn inbred line Hi38 (gene, brittle-1), which was developed by Dr. Brewbaker in Hawaii (Brewbaker, unpublished). G24 is one of the RILs derived from a cross between Hi34 and Ki14 (Moon *et al.*, 1999). The field experiments were under natural infestations for corn leaf aphid at a time that predator populations appeared to be abnormally low (Brewbaker, unpublished).

Two visual rating methods were adopted in the present study for the evaluation of resistance. The first rating was based on the degree of infestation in the whorl during tassel emergence. The second rating was based on infestation of ears covered with shootbags about 14 days after pollination (DAP). About ten plants in each plot were rated for both visual rating methods on 1 to 5 scales as follows: 1, no aphids; 2, light (lower than 50 aphids); 3, moderate (50 to 200 aphids); 4, heavy (200 to 500 aphids); and 5, severe (more than 500 aphids).

Generation mean analysis was conducted under the traditional assumptions of no epistasis or linkage. Additive and dominance genetic variance (σ_A^2 and σ_D^2) and environmental variance (σ_E^2) were estimated following Warner (1952), in which $\sigma_A^2 = 2\sigma_{F2}^2 - (\sigma_{B1}^2 + \sigma_{B2}^2)$, $\sigma_D^2 = \sigma_{F2}^2 - (\sigma_A^2 + \sigma_E^2)$, and $\sigma_E^2 = (\sigma_{P1}^2 + \sigma_{P2}^2 + 2\sigma_{F2}^2)/4$. The narrow and broad sense heritability (nH and bH) were calculated by σ_A^2/σ_E^2, and $(\sigma_A^2 + \sigma_D^2)/\sigma_E^2$ respectively.

Gene effects were based on a six-parameter model (Hayman, 1958, 1960). Estimates of gene effects (m = mean; a = additive; d = dominance or non-additive, aa, ad, dd = epistatic) were presented using the notation of Gamble (1962) and derived as

follows:

$m = F_2$

$a = B_1 - B_2$

$d = -(P_1)/2 - (P_2)/2 + F_1 - 4F_2 + 2B_1 + 2B_2$

$aa = -4F_2 + 2B_1 + 2B_2$

$ad = -(P_1)/2 + (P_2)/2 + 2F_1 + 4F_2 - 4B_1 - 4B_2.$

$dd = P_1 + P_2 + 2F_1 + 4F_2 - 4B_1 - 4B_2$

7.2.2. QTLs Analysis

A second set of maize germplasm was analyzed for aphid tolerance QTLs. One hundred RILs, the two parents (Hi34 and TZi17), and their F_1 were planted for the evaluations for tolerance to corn leaf aphid. The experiment was conducted at the Waimanalo Research Station of the Univ. of Hawaii, located at 21 N latitude on the island of Oahu. A randomized complete block design with two replications was planted in single row plots 2.5 m long with 0.75 m between-row spacing. The degree of corn leaf aphid infestation was rated on the first 5 plants of each row, using both tassel ratings and (pollination bag) covered ear ratings on 1 to 5 scales. The average ratings from both methods were used for QTL analysis.

The molecular map construction and statistical analysis followed the method described in Chapter 3.

7.3. Results

7.3.1. Generation Mean Analysis

Means and their standard errors for parental, F_1, F_2, and backcross generations are summarized in Table 7.1. Parent Hi38-71 was consistently highly resistant by both rating methods, while the other parent G24 was consistently susceptible. Means of F2 and BC populations segregated widely. Means of the F_1 were closer to the means of the susceptible parent, indicating that genes for resistance to corn leaf aphids in the resistant parent were mainly recessive.

Generation mean analysis provided estimates of six parameters (Table 7.2). Mean effects (m) were calculated simply as the mean of the F_2 progeny. Additive (a) effects, derived by comparing the BC1 and BC2 generations, were commonly significant and toward the susceptible parent G24. Dominance and epistasis effects (parameters aa, ad, dd) were commonly significant, suggesting a major influence of non-additive and gene interaction.

The phenotypic variance, averaged from the two rating data, contained 0.347 additive genetic variance, 0.372 dominance genetic variance, and 1.18 environmental variance. Mean heritability estimates were 18.2% for nH and 36.9% for bH respectively. The minimum number of effective factors was estimated by both Castle -Wright formulas, and averaged 1.47. The data suggests a major recessive gene may be responsible for resistance to corn leaf aphids in inbred Hi38-71.

Table 7.1. The corn leaf aphid ratings for parents Hi38-71 (P_1) and G24 (P_2), F_1, F_2, and backcross (B_1, B_2) generations.

Generations	Corn Leaf Aphid Rating (1=resistance, 5=sesceptible)		
	Tassels	Ears	Average
P_1	1.37 ± 0.24	1.13 ± 0.20	1.25 ± 0.15
P_2	3.58 ± 1.81	3.11 ± 1.10	3.34 ± 0.97
F_1	2.70 ± 1.35	3.47 ± 1.69	3.10 ± 0.79
F_2	2.57 ± 0.85	2.52 ± 1.47	2.55 ± 0.65
B_1	1.60 ± 0.25	2.00 ± 1.50	1.80 ± 0.50
B_2	3.00 ± 0.91	2.96 ± 1.29	2.98 ± 0.59

Table 7.2. Estimates of gene effects for resistance to corn leaf aphid from six generations of Hi38-71 (resistant) x G24 (susceptible)

Parameter	Tassel		Ear	
	Genetic effect	Standard error	Genetic effect	Standard error
m	2.57	0.93	2.52	1.21
a	1.40	1.08	0.96	1.67
d	-0.83	4.50	1.18	6.05
aa	-1.09	4.28	-0.18	5.88
ad	-1.55	6.19	-1.82	8.67
dd	2.31	6.31	1.42	8.72

7.3.2. QTL analysis

The two parents (Hi34 and TZi17) differed significantly ($P<0.01$) for resistance to corn leaf aphids in both rating methods. The mean tassel rating and mean ear rating on a 1-5 scale for corn leaf aphids of TZi17 were 3.72 and 3.76, respectively, while the mean tassel and the mean ear rating for Hi34 were 2.36 and 2.54, respectively. The correlation coefficient between the means of tassel rating and ear rating was highly significant ($P<0.01$). The distribution of resistance to corn leaf aphid in the RILs departed significantly from a unimodal distribution (Figure 7.1). Both the normal distribution method (Brewbaker, 1995, 1999) and the maximum likelihood estimate (Appendix 3) suggested that resistance to corn leaf aphid was controlled by a major gene.

Single factor analysis (SAS GLM) identified several genome regions associated with resistance to corn leaf aphids (Table 7.3). The most highly correlated chromosome marker was *bnl12.09*, located on the short arm of chromosome 2 ($F=14.165$, $R^2 = 0.143$). The data suggest that a major QTL was located in this genome region.

Composite interval mapping revealed a QTL for resistance with a peak LOD score of 8.2 near the marker *bnl12.09* on the short arm of chromosome 2 (Figure 7.2). This QTL was traced back to Antigua 2D, from which Hi34 was bred. Twenty-eight pairwise epistatic interactions ($P<0.01$) for resistance to corn leaf aphids among the markers were also detected by the Epistat (Chase *et al.*, 1997). Four of these pair-wise interactions involved marker *bnl12.09*. The detected QTL for resistance to corn leaf aphids, designated *aph2* in the current study, may also interact with other loci in conferring resistance to corn leaf aphids (Lu and Brewbaker, 1999).

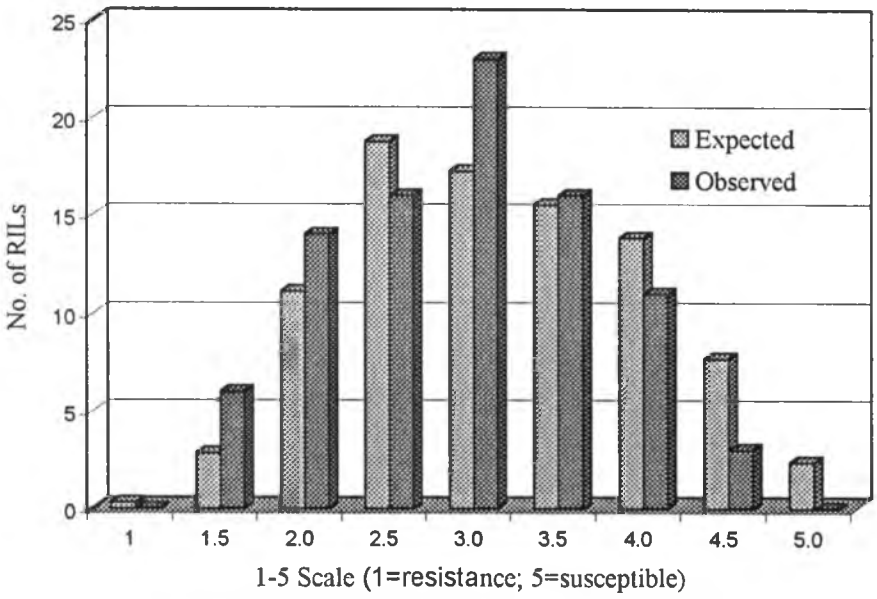

Figure 7.1. Mean aphid resistance ratings for ear and tassel data of 91 RILs derived from Hi34 x TZi17 in 1998 at Waimanalo, HI (2.4 for Hi34, 3.7 for Tzi17).

Table 7.3. Loci significantly associated with resistance to corn leaf aphid from single factor analyses in 100 RILs (Hi34 x TZi17) tested at the Waimanalo Research Station in 1998.

Locus	Bin†	F(1, n-2)	P
asg75	1.04	5.070	0.027
csu92	1.06	7.002	0.009
bnl8.45	2.01	4.052	0.047
npi239	2.01	10.972	0.001
bnl12.09	2.04	14.165	0.000
umc31	2.08	4.052	0.047
bnl6.25	5.01	10.286	0.002
npi409	5.01	5.608	0.020
umc124	8.03	5.348	0.023

†: Bin locations are designed by an X.Y code, where X is the linkage group containing the bin and Y is the location of the bin within the linkage group (Gardiner *et al.*, 1993).

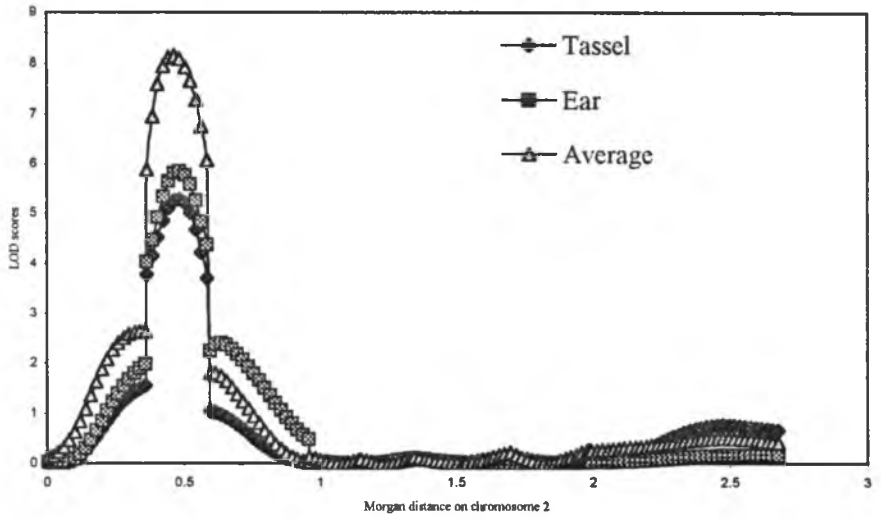

Figure 7.2: LOD scores of the region around the gene, *aph2* , for resistance to corn leaf aphid on chromosome 2

7.4. Discussions.

A single recessive gene appeared to be associated with the corn leaf aphid resistance in Hi38-71 in the current study. Chang and Brewbaker (1976) reported a monogenic recessive allele, *aph1*, for resistance to corn leaf aphids in AA8sh2 populations based on generation mean and diallel analyses. AA8sh2 is among the ancestors of Hi38-71 in a 70-generation pedigree (Brewbaker, unpublished).

A second major gene for aphid tolerance, designated *aph2*, was inferred from single factor analyses. A LOD peak of 8.2 was obtained at marker *bnl12.09* on chromosome 2 that explained 14.3% of the phenotypic variation. The highly significant pairwise interaction ($P<0.01$) indicated that *aph2* may also interact with other loci in affecting the resistance to corn leaf aphids. Thus *aph2* is rather critical in conferring resistance to corn leaf aphids both by itself or by the interaction with other alleles in the genome.

Pest resistance is usually controlled by multiple genes in nature. Three well documented insect resistance mechanisms are preference, antibiosis and tolerance (Painter 1951, 1968). Insect resistance in maize has been shown to be highly correlated with phytochemical composition, which includes nitrogen, fibers, phenolic acids, maysin, and 2, 4-dihydroxy-7-methoxy-1, 4-benzoxazin-3-one (DIMBOA) content, as well as leaf toughness. The mechanism of *aph2* in conferring aphid resistance may also involve phytochemical composition of corn plant, resulting in the inhibition of further infestations by aphids (Beck *et al.*, 1984; Bing *et al.*, 1991).

Both inbred Hi38-71 and Hi34 seem to confer major alleles for corn leaf aphid resistance, designated *aph1* for Hi38-71 and *aph2* for Hi34, respectively (Chang and Brewbaker, 1976; Lu and Brewbaker, 1999). The relationship between *aph1* and *aph2* is not clear at present, and studies are underway to determine their relationship.

Progeny testing is essential to the identification of QTLs conferring resistance to corn leaf aphids. The visual rating of covered ears by shootbag, in addition to the traditional visual tassel rating, seems to greatly increase the precision of QTL mapping. Additional experiments for the evaluation of resistance to corn leaf aphids in different environments will be necessary to confirm the *aph1* and *aph2* loci as defined in the current study, before they can be used in marker assisted selection or map-based cloning. Evaluations of aph1 resistance of Hi38-71 in upstate New York indicated that it is not susceptible to aphids in that location (Brewbaker, unpublished).

CHAPTER EIGHT

MOLECULAR MAPPING FOR RESISTANCE TO FALL ARMYWORM AND SUGARCANE BORER IN TROPICAL MAIZE

Abstract

The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), and sugarcane borer (SCB), *Diatraea saccharalis* (Fabricius), are major insect pests of maize in the Central America and the Southern USA. The genetic basis for resistance to FAW and SCB was investigated using DNA markers and progeny testing of maize recombinant inbred lines (RILs) developed from a cross between inbred lines Hi34 (resistant) and TZi17 (susceptible) by single seed descent without selection. FAW and SCB resistance was assessed by leaf damage ratings after artificial infestation in the field. Seven and four quantitative trait loci (QTLs) were identified for resistance to FAW and SCB, respectively.

8.1. Introduction

The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), and sugarcane borer (SCB), *Diatraea saccharalis* (Fabricius), are major insect pests of maize, *Zea mays* L., in Central America and the Southern USA. Larvae of both species feed extensively on the leaves and other above ground portions of corn at all stages, although the most serious damage occurs at the mid-whorl stage (Cruz, 1980). Extensive larval feeding by FAW or

SCB at whorl stage can substantially reduce grain yield (Hinderliter, 1983; Williams and Davis, 1990).

Field experiments have shown that antibiosis is the most important mechanism of resistance to FAW and SCB (Williams *et al.*, 1995), although resistant germplasm often possesses a combination of the three mechanisms of pest resistance - non-preference, antibiosis, and tolerance (Painter, 1968). Antibiosis can be evaluated by development of the insect population or by feeding damage, although visual damage is more subjective. Leaf damage ratings (LDR) for evaluation of antibiosis have been effective in breeding corn by visually selecting plants artificially infested with fall armyworm and sugarcane borer (Williams, 1989). At CIMMYT, germplasm conferring resistance against a number of species of corn borers, including SCB, was combined to form a multiple-borer-resistant (MBR) population (Smith *et al.*, 1989).

Resistance for FAW and SCB appeared to be polygenically controlled and thought to involve primarily additive gene action (Hinderliter, 1983). Most of the resistant maize genotypes were identified in germplasm originating from the Caribbean Islands (Ellas, 1970). Williams (1989) reported that general combining ability (GCA) for resistance to FAW was the most significant source of variation, while specific combining ability (SCA) was non-significant, based on diallel analysis of 8 corn inbreds. Thome *et al.* (1992) drew a similar conclusion with SCB resistance by evaluating 10 maize inbred lines for SCB resistance (five resistant and three susceptible CIMMYT lines, two public lines) in a diallel series. The success of S1 recurrent selection in the improvement of FAW resistance and

SCB resistance also suggested additive gene action (Khairallah *et al.*, 1996). Recent evidence supports a significant contribution of SCA for FAW resistance (Viana *et al.*, 1996; Williams *et al.* 1995). Breeding for resistance to FAW and SCB is thus laborious and time-consuming. It requires recurrent selection for four to five cycles of infestation to recover desirable resistance, and requires insect mass-rearing facilities.

The resolution of quantitative traits into Mendelian genes (quantitative trait loci or QTLs) can facilitate the understanding of the host-resistance mechanism. QTL mapping is also the first step toward marker-assisted selection procedure. Khairallah *et al.* (1996) and Bohn *et al.* (1996, 1997) identified 10 QTLs conferring resistance to SCB by applying the method of composite interval mapping. Pleiotropic QTL were also found for multiple resistance to European corn borer (ECB), Southwestern corn borer (SWCB) and SCB. No reports are available concerning QTL mapping results for resistance to FAW.

Many studies have identified and characterized QTLs related to a wide range of agronomic characters such as grain yield, grain quality and stress tolerance. Quantitative traits are affected by many genes, and each gene replacement may have effects on other genes affecting the same or different traits. Based on this perspective, epistasis should be considered in studying the inheritance of quantitative traits (Li *et al.*, 1995). Little evidence for epistasis has been reported from molecular marker-based studies of plant insect resistance traits, although epistasis has been well documented in agronomic characters such as grain yield and its components in rice (Li *et al.*, 1995; Yu *et al.*, 1997).

In this study we investigated the genetic basis of resistance to FAW and SCB in maize by means of QTL analyses using DNA markers and progeny testing of maize recombinant inbred lines (RILs). Our objectives were to (i) estimate the number, chromosomal positions, and genetic effects of QTL for resistance to FAW, and (ii) evaluate epistasis associated with resistance to FAW and SCB.

8.2. Materials and Methods

8.2.1. Agronomic Trials

The field experiments with artificial infestation by sugarcane borer and fall armyworm were planted at Poza Rica, Mexico, in a tropical environment, 60m elevation, 20.34^{0}N latitude. Two trials with fall armyworm infestations were conducted in the winter (December through April) and summer (April through August) of 1997. One trial with SCB infestations was conducted in the winter of 1997. Both winter trials included 100 RILs and the two parents, while the summer trial included 88 RILs and the two parents. The experimental design was a randomized complete block design with two replications and single-row plots 5m long.

Every plant in each trial was artificially infested with about 30 larvae at the 6-8 leaf (mid-whorl) stage. Larvae were mixed with corn-cob grits and placed in the plant whorl with a mechanical larval dispenser (Wiseman *et al.*, 1980).

Visual leaf damage ratings for FAW were recorded on ten plants in each plot after 90 days on the following scale: 0 = slight pinhole damage; 1 = pinholes on at least 2 leaves; 2 = shot holes and a few elongated lesions; 3 = shot holes and several elongated lesions; 4 = many elongated lesions; 5 = many elongated lesions and a few portions eaten away; 6 = many elongated lesions and several portions eaten away; 7= many elongated lesions, portions eaten away, and damage in whorl; 8 = many elongated lesions, portion eaten away, and whorl destroyed; and 9 = plant dying or dead. Visual leaf damage ratings for SCB were recorded on ten plants in each plot on the following scale: 0 = no damage or few pinholes to leaf; 1= pinholes on at least two leaves; 2 = few shot holes on few leaves; 3 = several leaves with shot holes; 4 = several leaves with shot holes and a few long lesions (<2.5cm); 5 = several leaves with long lesions (<2.5cm); 6 = several leaves with long lesions (>2.5cm); 7 = long lesions (>2.5cm) common on half of the leaves; 8 = long lesions (>2.5cm) common on 1/2 to 2/3 of leaves; 9 = most leaves with long lesions (>2.5cm) or plant dead.

8.2.2. Statistical Analyses

QTLs were identified by using QTL Cartographer 1.12f (Basten *et al.*, 1997) for composite interval mapping (Zeng, 1994). Single-factor analyses of variance were computed for each locus-trait combination by using the GLM procedure in SAS (SAS Institute, 1989). Two-factor analyses of variance were also computed to determine main effects of the two loci plus their interaction. Pre-selection techniques were used to reduce

the number of factors to be considered. Main effects of loci were considered for model building and for all possible two-locus interactions of all marker loci if main effects of loci were significant at $P < 0.05$.

8.3. Results

8.3.1. QTL Analyses for FAW Resistance

The average leaf damage ratings (LDR) for FAW were 5.2 for parent Hi34 and 6.9 for parent TZi17 from the combined data of winter and summer trials. The overall LDR mean of the 100 RILs for FAW was 5.8, and data ranged from 4.6 to 7.0. Analysis of variance results indicated that variation among the RILs for FAW LDR was highly significant ($P<0.01$). Continuous variation was observed across the RIL populations, indicating several QTLs were responsible for resistance to FAW. Genotype x environment interactions were highly significant ($P<0.01$).

QTL analyses were performed using the 120 DNA marker loci and phenotypic data from the two environments. Identification of DNA marker loci linked to FAW resistance was conducted using single factor analysis of variance. Totals of 12 markers (winter) and 8 markers (summer) showed significant association with FAW resistance. These markers span 6 chromosomes, indicating several minor QTLs for FAW resistance.

Of the 2380 possible two-way interactions between the selected 20 markers and the other markers, we detected 106 significant ($P<0.05$) interactions for FAW resistance.

In both trials, one pair of marker loci that were closely linked to QTL but on different chromosomes were shown to interact significantly for FAW resistance.

Four putative QTL located on chromosomes 3, 4, 8 and 10 (Table 8.1) were found to significantly affect FAW resistance in the winter trials by the composite interval mapping method (QTL cartographer 1.12). We selected a total of 7 DNA markers (*npi232, csu29, csu25, mag1f03, umc102, umc45, and bnl3.04)* as cofactors. LOD scores ranged from 2.5 on chromosome 3 to 3.5 on chromosome 4. The latter QTL explained 9.9% of total phenotypic variance (Table 8.1). Altogether these four QTLs explained 30.1% of total phenotypic variance.

Three other putative QTL located on chromosomes 1, 2 and 9 (Table 8.1) were also identified for resistance to FAW in the summer trials. We selected a total of nine DNA markers (*umc18, umc113, bnl5.62, csu95, csu36, npi232, bnl8.01, npi451, and asg75)* as cofactors. The highest LOD score was 4.7 for the QTL on chromosome 2 and this QTL explained 6.7% of total phenotypic variance. These three QTLs altogether explained about 19.2% of total phenotypic variance. QTL x environment interaction was highly significant in this case, as all putative QTLs were inconsistent across the two environments.

8.3.2. QTL Analyses for SCB Resistance

The average leaf damage rating (LDR) for SCB was 5.1 for parent Hi34 and 7.3 for parent TZi17 from the winter 1997 trial. The overall LDR mean of all 100 RILs for

Table 8.1. Composite interval mapping for FAW resistance. Parameters of QTL effects were estimated from the phenotypic means of 100 recombinant inbred lines from cross Hi34 x TZi17 evaluated at one tropical location in two growing season.

Bin*	Marker interval	LOD score	R^2 (%)	Cluster resistance traits in the bin	Gene or QTL for the traits
FAW resistance in winter 1997					
3.04	npi220-umc102	2.5	4.0	*Puccinia sorghi*	*rp*3
				Gibberella stalk rot	*qgsr*2
				Wheat streak mosaic virus	*wsm*2
				Maize mosaic virus	*mv*1
				European corn borer	*q2ecb*8
4.04	bnl5.46-mag1f03	3.5	9.9	Gibberella stalk rot	*qgsr*3
				Gibberella stalk rot	*qgsr*8
8.01	csu29-umc123	3.4	9.3		
10.05	npi232-umc29	3.0	6.9	European corn borer	
				Southwestern corn borer	
				Wheat streak mosaic virus	*wsm*3
				Gibberella stalk rot	*qgsr*5
				Gibberella stalk rot	*qgsr*10
FAW resistance in summer 1997					
1.01	bnl5.62-umc164	3.6	4.4		
2.05	csu110-csu50	4.7	6.7	Gibberella stalk rot	*qgsr*7
9.02	umc113-bnl8.17	3.6	7.1	Maysin content	*qmaysin*5

* Bin locations are designed by an X.Y code, where X is the linkage group containing the bin and Y is the location of the bin within the linkage group (Gardiner *et al.*, 1993)

SCB was 6.2, with range from 2 to 8.6. Analyses of variance indicated that variation among the RILs for SCB was highly significant ($P<0.01$). Continuous variation was observed across the RIL populations, suggesting that several QTLs were responsible for resistance to SCB.

A total of 11 DNA markers showed significant correlation with SCB resistance using single factor analyses of the 120 DNA marker loci and LDR data for winter of 1997. Five of the 11 markers were located on chromosome 1 and three were on chromosome 3. These 11 marker loci were tested for di-genic interactions. Of all the 1309 possible two-way ANOVAs between these 11 marker loci and all other marker loci, we detected 71 significant interactions ($P<0.05$) for SCB resistance. Significant interactions characterized one pair of marker loci closely linked to two QTLs for SCB resistance

A total of 14 DNA markers (*npi238, umc199, umc113, umc89, csu36, umc166, umc72, npi285, bnl5.62, npi220, umc107, npi297, csu46, and csu136)* were selected as cofactors for composite interval mapping. Three putative QTL located on chromosomes 1 and 3 were found to affect SCB resistance significantly (Table 8.2). LOD scores for QTLs on chromosome 1 were 2.5 and 5.2, accounting for 5.4% and 9.7% of the total phenotypic variance respectively. LOD score for the QTL on chromosome 3 was 3.6, explaining 4.9% of the total phenotypic variance.

Table 8.2. Composite interval mapping for SCB resistance. Parameters of QTL effects were estimated from the phenotypic means of 100 recombinant inbred lines from cross Hi34 x TZi17 evaluated at one tropical location in winter 1997.

Bin*	Marker Interval	LOD score	R^2 (%)	Cluster resistance traits in the bin	Gene or QTL for the traits
1.05	umc167-umc166	2.5	5.4	*Stewart's Wilt*	*sw*1
				Maize streak virus	*msv*1
				Exserohilum turcicum	*ht*4
				Exserohilum carbonum	*hm*1
				Maysin content:	*qmaysin*2
1.11	umc104-npi 238	5.2	9.7	European corn borer	
				Southwestern corn borer	
3.09	umc199-bnl12.30	3.6	4.9		

* Bin locations are designed by an X.Y code, where X is the linkage group containing the bin and Y is the location of the bin within the linkage group (Gardiner *et al.*, 1993).

8.3.3. Clustering of Resistance QTLs

Based on a literature review, McMullen and Simcox (1995) reported that the majority of disease and insect resistance genes or QTLs occur in clusters. Five of the seven QTLs for FAW resistance and two of the three QTLs for SCB resistance identified in this study were located in the same chromosomal bins as genes or QTLs for resistance to other diseases and insects (Table 1 and Table 2). These chromosomal bins contained resistance factors against some fungal diseases (Gibberella stalk rot, *Puccinia sorghi* and *Puccinia polysora, Stewart's Wilt, Exserohilum turcicum* and *Exserohilum carbonum*), virus (maize mosaic virus, maize streak virus, wheat streak virus) and insects (European corn borer, southwestern corn borer). No information is yet clearly available on the functional relationship between genes and QTLs located in the same bin for different maize disease and pest.

The QTL for resistance to FAW located on bin 9.02 was clustered with *qmaysin5* locus, which contributes toward maysin content and antibiosis for several insect resistances (Byrne *et al.*, 1996). The QTL for resistance to SCB located on bin 1.05 was also clustered with *qmaysin2*. These results suggest that maysin content maybe involved in the antibiosis of SCB and FAW resistance.

8.4. Discussion

Khairallah et al. (1996) and Bohn et al. (1996, 1997) identified 10 QTLs for SCB resistance in one population of RILs based on CML131 x CML 67 in three environments. CML 67 is related by descent to Hi34 of the present study (origin in variety Antigua). The QTL located in bin 1.11 for SCB resistance identified in this study is consistent with their reports (Khairallah *et al.*, 1996; Bohn *et al.*, 1996, 1997). This indicates that this QTL provides a common genetic basis for resistance to SCB for in these experiment, although the genetic materials and experimental environments were different. This QTL for SCB resistance and that in bin 10.05 for FAW resistance were also closely linked with genes for resistance to European corn borer and southwestern corn borer. These genome regions may have a pleiotropic effect for resistance to multiple borers and FAW, and may involve similar biochemical pathways.

The complexities of analyzing QTL inheritance and expression patterns raise questions as to practical approaches to marker-assisted selection (Schön *et al,* 1993). One potential complication is genotype-environment interaction, with some regions only becoming "active" under certain conditions. It is critical to ascertain which region are the most important in enhancing the trait of interest. These should be regions with high stability across environments, such as the QTLs located in bin 1.11 for SCB resistance. Regions with cluster genes or with pleiotropic effects for different resistance may also be considered to improve the efficiency of marker assisted selection.

In summary, seven and three QTLs were identified for resistance to FAW and SCB respectively. Five of the seven QTLs for FAW resistance and two of the three QTLs for SCB resistance identified in this study appear to be incorporated in clusters of resistance genes. One of the three QTLs identified in this study for SCB resistance corresponded to an existing characterized QTL (Bohn et al., 1996, 1997), while the others are first reported here. The QTLs for FAW and SCB resistance located in the same chromosomal bins with maysin QTLs suggested the importance of the phytochemical basis for insect resistance (Bergvinson *et al.*, 1996).

Appendix A. Response of RILs derived from Hi34 x TZi17 for disease resistance*

Pedigree	Maize Streak Virus			Head Smut	Common Rust			
	IITA 92	IITA 93	Mean	S. Africa 94	CIMMYT 93	HI 93	HI 97	Mean
	1-9	1-9	1-9	1-9	1-9	1-9	1-9	1-9
Hi3	7.0	7.2	7.1	5.8	3.5	3.3	4.9	3.9
TZi	2.4	2.2	2.3	2.4	6.0	7.2	7.5	6.9
F1				5.5				
I 1	2.0	9.0	5.5	9.0	7.0	3.0	6.5	5.5
I 2	3.0	8.0	5.5	2.0	-	6.5	-	6.5
I 3	7.0	3.0	5.0	6.0	3.0	6.5	5.2	4.9
I 4	7.0	-	7.0	3.0	7.5	7.0	6.3	6.9
I 5	3.0	-	3.0	3.0	4.0	4.5	4.2	4.2
I 6	2.0	3.0	2.5	1.0	7.0	7.5	7.0	7.2
I 7	2.0	2.0	2.0	2.0	6.5	8.0	7.0	7.2
I 8	2.0	4.0	3.0	4.0	-	7.0	3.2	5.1
I 9	7.0	6.0	6.5	9.0	-	8.5	7.0	7.7
I 10	2.0	3.0	2.5	2.0	7.5	8.0	6.7	7.5
I 11	7.0	8.0	7.5	1.0	7.0	6.5	6.2	6.6
I 12	2.0	6.0	4.0	5.0	8.0	7.5	7.2	7.6
I 13	8.0	6.0	7.0	3.0	3.0	5.5	5.2	4.6
I 14	7.0	6.0	6.5	4.0	6.5	6.0	5.7	6.1
I 15	2.0	1.0	1.5	6.0	7.0	7.5	-	7.2
I 16	5.0	6.0	5.5	6.0	3.5	5.5	5.2	4.7
I 17	3.0	6.0	4.5	4.0	8.5	8.0	8.5	8.3
I 19	3.0	2.0	2.5	5.0	6.5	8.0	3.3	5.9
I 20	2.0	2.0	2.0	3.0	4.0	6.5	-	5.2
I 21	2.0	2.0	2.0	1.0	6.0	7.0	5.8	6.3
I 22	2.0	2.0	2.0	7.0	6.5	6.0	6.0	6.2
I 24	2.0	6.0	4.0	7.0	6.5	6.5	5.8	6.3
I 25	2.0	2.0	2.0	2.0	7.0	8.0	6.0	7.0
I 26	2.0	7.0	4.5	5.0	4.0	5.5	3.7	4.4
I 27	2.0	2.0	2.0	2.0	3.0	5.5	3.0	3.8
I 28	7.0	2.0	4.5	4.0	2.0	6.5	2.8	3.7
I 29	4.0	7.0	5.5	2.0	7.0	5.5	3.5	5.3
I 30	2.0	2.0	2.0	3.0	-	6.5	-	6.5
I 31	2.0	7.0	4.5	7.0	6.5	6.5	5.0	6.0
I 32	2.0	3.0	2.5	1.0	6.0	5.5	-	5.7
I 33	2.0	2.0	2.0	2.0	2.5	6.0	4.8	4.4
I 34	2.0	2.0	2.0	3.0	5.0	6.0	5.3	5.4
I 36	4.0	4.0	4.0	6.0	7.0	8.0	7.3	7.4
I 38	6.0	2.0	4.0	4.0	5.0	8.0	4.3	5.8
I 39	-	-	-	-	7.0	-	4.3	5.6
I 40	2.0	2.0	2.0	1.0	3.0	6.0	4.0	4.3

Appendix A. Cont.

Pedigree	Maize Streak Virus			Head Smut	Common Rust			
	IITA 92	IITA 93	Mean	S. Africa 94	CIMMYT 93	HI 93	HI 97	Mean
	1-9	1-9	1-9	1-9	1-9	1-9	1-9	1-9
I 41	2.0	2.0	2.0	1.0	6.5	7.0	4.8	6.1
I 42	3.0	2.0	2.5	7.0	7.0	7.5	6.2	6.7
I 44	2.0	2.0	2.0	3.0	6.5	8.0	4.7	6.4
I 45	6.0	7.0	6.5	3.0	2.5	7.5	3.2	4.4
I 46	2.0	2.0	2.0	2.0	3.5	6.5	3.2	4.4
I 47	2.0	2.0	2.0	2.0	7.0	6.5	5.0	6.2
I 48	2.0	7.0	4.5	3.0	3.0	5.0	5.7	4.6
I 49	2.0	2.0	2.0	3.0	6.0	8.5	6.8	7.1
I 50	2.0	4.0	3.0	3.0	8.0	8.0	7.2	7.7
I 51	2.0	7.0	4.5	3.0	4.5	6.5	5.0	5.3
I 52	7.0	7.0	7.0	9.0	6.5	7.0	5.2	6.3
I 53	2.0	8.0	5.0	3.0	7.0	7.5	5.5	6.7
I 54	5.0	2.0	3.5	4.0	2.5	6.0	4.5	4.3
I 55	5.0	5.0	5.0	3.0	8.5	8.0	7.8	8.1
I 56	4.0	2.0	3.0	6.0	6.0	6.0	6.3	6.1
I 57	7.0	3.0	5.0	6.0	7.0	7.0	6.7	6.9
I 58	6.0	8.0	7.0	2.0	3.0	5.5	3.5	4.0
I 59	6.0	8.0	7.0	6.0	7.0	7.0	5.2	6.4
I 60	2.0	2.0	2.0	1.0	6.5	7.0	5.7	6.4
I 62	5.0	8.0	6.5	2.0	2.5	4.0	3.0	3.2
I 63	2.0	7.0	4.5	6.0	7.0	6.5	3.8	5.8
I 64	2.0	2.0	2.0	4.0	7.5	7.5	6.0	7.0
I 66	7.0	4.0	5.5	4.0	6.0	6.0	3.8	5.3
I 67	2.0	7.0	4.5	5.0	4.0	6.0	4.0	4.7
I 68	4.0	2.0	3.0	6.0	6.5	6.0	4.3	5.6
I 69	2.0	3.0	2.5	3.0	4.0	5.0	4.2	4.4
I 70	2.0	8.0	5.0	9.0	4.0	6.0	4.8	4.9
I 71	2.0	2.0	2.0	4.0	5.0	7.5	4.7	5.7
I 72	7.0	2.0	4.5	1.0	3.0	6.5	5.0	4.8
I 73	2.0	8.0	5.0	4.0	7.0	8.0	6.5	7.2
I 74	5.0	8.0	6.5	2.0	3.0	5.5	4.3	4.3
I 75	3.0	8.0	5.5	9.0	9.0	8.5	8.0	8.5
I 76	3.0	3.0	3.0	3.0	3.0	7.0	3.2	4.4
I 77	2.0	7.0	4.5	2.0	3.0	6.0	5.8	4.9
I 78	3.0	7.0	5.0	6.0	8.5	9.0	7.0	8.2
I 79	7.0	7.0	7.0	5.0	6.0	5.5	7.0	6.2
I 80	2.0	7.0	4.5	5.0	3.5	6.0	-	4.7
I 81	2.0	3.0	2.5	3.0	4.0	7.0	-	5.5
I 83	3.0	6.0	4.5	2.0	6.0	6.5	5.2	5.9
I 84	-	-	-	-	8.0	-	-	8.0

Appendix A. Cont.

Pedigree	Maize Streak Virus			Head Smut	Common Rust			
	IITA 92	IITA 93	Mean	S. Africa 94	CIMMYT 93	HI 93	HI 97	Mean
	1-9	1-9	1-9	1-9	1-9	1-9	1-9	1-9
I 85	5.0	3.0	4.0	3.0	6.5	7.5	-	7.0
I 86	2.0	8.0	5.0	5.0	2.5	7.0	5.2	4.9
I 87	3.0	2.0	2.5	2.0	3.0	7.0	3.2	4.4
I 89	2.0	2.0	2.0	2.0	7.0	8.0	6.0	7.0
I 91	8.0	8.0	8.0	2.0	8.0	6.0	-	7.0
I 92	2.0	7.0	4.5	4.0	3.0	7.5	-	5.3
I 93	2.0	3.0	2.5	5.0	5.0	6.0	6.7	5.9
I 94	2.0	7.0	4.5	4.0	4.5	3.5	-	4.0
I 95	6.0	8.0	7.0	3.0	6.0	6.5	5.8	6.1
I 96	2.0	2.0	2.0	2.0	7.5	7.0	-	7.2
I 97	-	-	-	-	6.0	-	-	6.0
I 98	4.0	2.0	3.0	5.0	4.0	8.0	-	6.0
I 99	3.0	7.0	5.0	8.0	6.0	6.5	6.0	6.2
I 100	6.0	2.0	4.0	6.0	4.0	6.0	4.8	4.9
I 101	6.0	7.0	6.5	4.0	6.5	7.5	6.2	6.7
I 102	-	-	-	-	-	-	5.3	5.3
I 103	3.0	3.0	3.0	7.0	5.5	7.0	6.7	6.4
I 104	4.0	2.0	3.0	5.0	4.0	7.0	5.5	5.5
I 105	6.0	8.0	7.0	4.0	3.0	6.0	3.8	4.3
I 106	4.0	8.0	6.0	1.0	7.5	6.0	-	6.6
I 107	-	-	-	-	-	-	8.0	8.0
I 108	-	-	-	-	-	-	5.7	5.7
I 109	-	-	-	-	-	-	7.7	7.7
I 110	-	-	-	-	-	-	6.3	6.3
Count	18.0	18.0	18.0	18.0	19.0	18.0	16.0	24.0
Mean	3.9	4.9	4.4	4.0	5.2	6.7	5.8	6.0
STD	1.9	2.7	1.9	1.9	1.7	1.0	1.2	1.1
CV(%)	48.2	55.3	42.2	47.0	33.0	15.6	21.3	17.6

*. MSV was recorded at IITA (Nigeria) in 1992 and 1993, head smut at Greytown, South Africa, and common rust at CIMMYT (Mexico) and Waimanalo, HI.

Appendix B. Response of RILs derived from Hi34 x TZi17 for insect resistance*

Pedigree	Corn Leaf Aphid			Fall Armyworm			Sugarcane Borer
	Tassel	Ear	Mean	CIMMYT 97	CIMMYT 98	Mean	CIMMYT 97
	1-5	1-5	1-5	1-9	1-9	1-9	1-9
Hi34	2.4	2.5	2.5	5.6	5.7	5.7	5.1
TZi17	3.7	3.8	3.8	7.3	6.4	6.9	7.3
F1	4.3	3.7	4.0				
I 1	2.7	2.9	2.8	5.8	6	5.9	-
I 2	-	-	-	-	-		-
I 3	3.3	3.3	3.3	6.1	6.5	6.3	8.1
I 4	3.5	2.5	3	6.3	5.6	5.95	6.3
I 5	2.3	3.1	2.7	6.4	5	5.7	8.2
I 6	2.8	1.3	2.1	6.5	3	4.75	6.3
I 7	2.2	2.8	2.5	5.9	6.2	6.05	7.4
I 8	3.3	2.2	2.7	4.7	-	4.7	8.6
I 9	4.2	3	3.6	5.7	-	5.7	-
I 10	2	2.5	2.6	6.9	-	6.9	5.6
I 11	5	3.2	4.1	5.7	5.3	5.5	7.7
I 12	3.8	2.7	3.3	5.5	5.2	5.35	7.8
I 13	4	3.6	3.8	6.2	4.9	5.55	5.9
I 14	4.4	2	3.2	6.1	5.6	5.85	8.4
I 15	-	-	-	5.8	3.9	4.85	4.3
I 16	4.4	2	3.2	6.4	5.8	6.1	6.1
I 17	4.3	3.1	3.7	5.5	4.1	4.8	4.8
I 19	1.8	1.4	1.6	5.3	3.9	4.6	5.8
I 20	2.6	4.5	3.6	6.6	-	6.6	-
I 21	3.2	1.9	2.6	6	5	5.5	4.4
I 22	1.4	1	1.2	4.7	7	5.85	5.5
I 24	2.3	2.5	2.4	5.8	3.3	4.55	7.8
I 25	1.9	2.1	2	6.3	4.2	5.25	8.2
I 26	1.7	1.4	1.6	6.5	6	6.25	8
I 27	1.1	1.3	1.2	5.8	4.7	5.25	6
I 28	1.2	2.7	2	5.9	4.2	5.05	7.2
I 29	1.4	3.6	2.5	5.9	-	5.9	5
I 30	-	-	-	6.5	-	6.5	6.6
I 31	4.2	3	3.6	5	6.9	5.95	5.2
I 32	3.9	1.9	2.9	6	4.6	5.3	6
I 33	1.5	1.8	1.7	6	-	6	5.8
I 34	3.8	4.3	4.1	6.2	4.4	5.3	6.5
I 36	2.3	2.5	2.4	6.4	4.9	5.65	8
I 38	1.8	2.8	2.3	6.5	5.9	6.2	7.5
I 39	2.8	3.3	3.1	5.7	4.2	4.95	4.6
I 40	2.5	3.3	3.1	6.2	5	5.6	6.5

Appendix B. Cont.

Pedigree	Corn Leaf Aphid			Fall Armyworm			Sugarcane Borer
	Tassel	Ear	Mean	CIMMYT 97	CIMMYT 98	Mean	CIMMYT 97
	1-5	1-5	1-5	1-9	1-9	1-9	1-9
I 41	2.2	2.4	2.3	5.2	-	5.2	7.9
I 42	2.0	2.0	2.0	4.6	5.9	5.3	8.0
I 44	2.4	4.5	3.5	6.6	4.5	5.6	6.5
I 45	-	-	-	6.0	5.9	6.0	-
I 46	4.4	3.4	3.9	5.3	4.6	5.0	3.6
I 47	2.5	2.4	2.5	5.9	6.3	6.1	5.4
I 48	2.7	2.1	2.4	5.3	4.7	5.0	5.4
I 49	2.0	4.3	3.2	5.9	5.6	5.8	8.0
I 50	3.0	3.8	3.4	6.8	4.5	5.7	8.2
I 51	2.6	2.6	2.6	4.8	4.5	4.7	5.8
I 52	2.9	2.3	2.6	5.9	5.3	5.6	4.1
I 53	2.5	3.4	3.0	4.6	3.8	4.2	8.4
I 54	1.4	3.0	2.2	6.7	3.8	5.3	5.5
I 55	3.3	3.6	3.4	6.2	5.0	5.6	8.0
I 56	3.5	3.0	3.3	6.2	3.6	4.9	5.9
I 57	1.4	3.8	2.6	5.2	5.9	5.6	6.0
I 58	4.1	1.2	2.7	4.7	6.4	5.6	5.1
I 59	3.6	2.7	3.2	5.9	5.1	5.5	5.6
I 60	1.7	2.1	1.9	5.4	3.7	4.6	7.1
I 62	3.2	2.3	2.8	5.6	-	5.6	-
I 63	1.2	1.9	1.6	6.5	3.8	5.2	6.8
I 64	4.6	3.0	3.8	6.2	5.7	6.0	7.1
I 66	1.7	4.3	3.0	5.9	4.7	5.3	7.3
I 67	-	-	-	5.2	-	5.2	3.5
I 68	3.4	2.7	3.1	4.7	7.5	6.1	6.6
I 69	5.0	1.7	3.4	6.0	6.7	6.4	7.3
I 70	3.2	3.2	3.2	4.8	6.1	5.5	5.2
I 71	1.8	2.6	2.2	5.6	5.9	5.8	4.8
I 72	4.1	4.1	4.1	6.4	5.7	6.1	5.4
I 73	4.6	2.8	3.7	5.0	4.2	4.6	6.8
I 74	2.9	2.2	2.6	5.2	5.0	5.1	6.4
I 75	1.9	2.3	2.1	6.1	4.9	5.5	6.4
I 76	2.0	1.8	1.9	6.2	6.6	6.4	5.3
I 77	2.0	2.3	2.2	6.1	6.9	6.5	5.3
I 78	3.1	2.5	2.8	5.9	5.1	5.5	7.0
I 79	2.6	2.8	2.7	5.1	6.1	5.6	5.0
I 80	-	-	-	4.8	-	4.8	5.8
I 81	2.1	2.7	2.4	6.3	5.3	5.8	4.4
I 83	1.5	2.7	2.1	6.1	4.3	5.2	7.0
I 84	2.6	3.9	3.3	6.6	-	6.6	-

Appendix B. Cont.

Pedigree	Corn Leaf Aphid			Fall Armyworm			Sugarcane Borer
	Tassel	Ear	Mean	CIMMYT 97	CIMMYT 98	Mean	CIMMYT 97
	1-5	1-5	1-5	1-9	1-9	1-9	1-9
I 85	1.6	2.1	1.9	6.0	-	6.0	-
I 86	3.7	3.4	3.6	5.5	3.9	4.7	6.3
I 87	3.8	2.9	3.4	6.1	6.0	6.1	5.9
I 89	4.4	3.6	4.0	5.3	4.3	4.8	5.0
I 91	1.0	1.4	1.2	4.8	4.7	4.8	5.0
I 92	-	-	-	5.1	7.2	6.2	8.5
I 93	3.7	2.1	2.9	7.0	5.0	6.0	4.0
I 94	3.5	1.9	2.7	6.1	-	6.1	7.8
I 95	2.7	3.5	3.1	6.4	6.0	6.2	7.5
I 96	1.2	1.6	1.4	6.2	6.3	6.3	7.5
I 97	1.1	1.2	1.2	5.4	-	5.4	2.3
I 98	4.6	3.0	3.8	5.0	5.8	5.4	7.7
I 99	-	-	-	4.9	-	4.9	6.0
I 100	3.1	2.5	2.8	5.3	4.8	5.1	5.1
I 101	1.0	2.3	1.7	5.9	-	5.9	3.9
I 102	2.2	1.6	1.9	5.9	-	5.9	-
I 103	1.1	2.2	1.7	5.9	4.0	5.0	4.1
I 104	3.0	3.0	3.0	6.3	3.8	5.1	2.0
I 105	2.1	1.9	2.0	5.9	3.3	4.6	5.4
I 106	-	-	-	4.7	-	4.7	6.4
I 107	3.0	2.4	2.7	5.3	4.8	5.1	6.8
I 108	2.3	2.0	2.2	5.9	-	5.9	-
I 109	3.1	2.1	2.6	5.2	6.6	5.9	4.8
I 110	1.5	1.0	1.3	5.9	4.0	5.0	8.0
Count	21.0	21.0	21.0	24.0	16.0	24.0	21.0
Mean	2.6	2.3	2.4	5.7	5.0	5.4	5.7
STD	1.2	0.7	0.9	0.6	1.2	0.6	1.8
CV(%)	0.5	0.3	0.4	0.1	0.2	0.1	0.3

*. Corn leaf aphid was recorded at Waimanalo, fall armyworm and sugarcane borer at CIMMYT (Mexico)

Appendix C: Maximum likelihood tests of mixed population model for presence of major QTLs conferring resistance to insects and diseases in RILs derived from Hi34 X TZi17.

Trait	Estimate of μ_{QQ}	Estimate of μ_{qq}	Likelihood ratio
MSV‡	2.65	5.58	14.45**
Head smut	2.65	5.60	3.87*
Common rust	6.15	7.14	5.62*
Corn leaf aphid	2.13	3.23	5.12*
Fall armyworm	5.21	6.16	0.78
Sugarcane borer	5.13	7.22	8.12**

‡ Means of resistant and susceptible are on 1-9 scale (1=resistant) except corn leaf aphid on 1-5 scale (1=resistant).

*, ** Significant deviation from unimodal distribution at $P < 0.05$ and $P < 0.01$.

Appendix D: Correlation coefficients among the traits for resistance to diseases and insects measured for 100 RILs derived from Hi34 x TZi17.

	MSV	Head smut	Common rust	Corn leaf aphid	FAW	SCB
MSV		0.205*	-0.050	0.087	-0.072	-0.015
Head smut			0.165	-0.006	-0.057	-0.102
Common rust				0.061	-0.053	0.131
Corn leaf Aphid					0.065	0.091
FAW						0.077

* Significant at P<0.05 (significant value was 0.195 at the 5%, 0.254 at the 1%).

Appendix E: Data of 117 RFLP and 4 SSR markers on 100 RILS (Hi34 x TZi17).
(A = Hi34 allele, B = TZi17 allele, - = other allele)

	RILs																			
DNA Marker	1 I1	2 I2	3 I3	4 I4	5 I5	6 I6	7 I7	8 I8	9 I9	10 I10	11 I11	12 I12	13 I13	14 I14	15 I15	16 I16	17 I17	18 I18	19 I19	20 I21
1 *asg30	A	B	A	A	B	B	B	B	A	B	A	A	A	B	B	A	B	A	B	B
2 *asg62	A	B	A	A	B	B	B	B	B	B	-	A	B	A	A	B	B	B	A	B
3 *asg75	A	B	A	A	B	-	B	B	A	B	A	A	A	B	B	B	B	A	B	B
4 *bnl10.24	B	B	A	B	A	A	A	-	A	-	A	B	A	B	B	B	B	A	A	A
5 *bnl12.09	B	-	B	B	B	A	A	A	A	B	B	A	-	B	A	-	-	A	B	B
6 *bnl12.30	B	B	B	A	B	B	A	A	A	A	B	A	A	-	A	A	B	A	A	-
7 *bnl12.30b	A	A	B	B	A	A	A	B	B	A	B	A	B	B	B	B	A	A	B	B
8 *bnl13.05	-	B	A	A	A	A	B	B	B	A	B	A	A	A	B	A	B	A	B	A
9 *bnl16.06	B	B	B	A	B	B	A	B	B	B	B	B	A	A	A	B	B	A	A	A
10 *bnl3.04	B	-	B	B	B	A	-	A	B	A	A	A	A	B	A	B	B	B	A	A
11 *bnl4.06	A	B	B	A	B	B	B	A	B	A	B	B	B	B	A	B	B	A	B	A
12 *bnl5.40	A	B	B	A	A	A	B	A	A	A	B	B	B	B	B	B	B	A	A	A
13 *bnl5.46	A	B	B	B	B	B	A	B	B	A	B	A	A	-	B	A	B	A	A	B
14 *bnl5.62	A	B	A	B	A	B	B	A	B	B	B	A	B	A	B	A	B	B	A	-
15 *bnl6.25	-	-	B	B	-	A	A	B	B	-	B	A	B	-	B	-	B	-	B	-
16 *bnl7.49	B	B	B	B	A	-	B	A	A	A	B	-	B	B	-	-	B	A	A	A
17 *bnl7.71	B	B	B	A	B	B	B	A	B	A	B	B	B	B	A	B	B	A	A	A
18 *bnl8.01	B	B	A	-	A	A	A	-	A	A	B	B	A	B	B	B	B	A	A	A
19 *bnl8.17	A	B	A	B	B	B	A	-	B	A	B	B	B	B	A	B	B	A	B	B
20 *bnl8.39	B	B	A	B	B	A	A	B	-	B	B	B	B	B	B	B	B	B	A	A
21 *bnl8.45	A	B	A	A	B	A	A	A	B	A	B	A	B	A	B	B	B	B	A	A
22 *csu110	A	B	A	A	B	B	B	A	B	A	A	A	B	B	B	B	B	B	A	B
23 *csu11	A	B	A	B	B	A	A	-	B	A	B	A	B	A	B	B	B	B	A	A
24 *csu12	A	B	B	B	B	A	A	B	A	-	-	-	B	B	-	B	B	A	A	B
25 *csu136	A	B	A	A	-	B	-	A	A	A	-	A	A	A	B	A	B	A	A	A
26 *csu13	-	B	-	B	A	B	A	B	A	B	A	A	B	A	A	A	B	A	B	B
27 *csu145	-	A	-	A	-	B	A	A	A	B	A	A	A	B	A	B	A	A	-	B
28 *csu146	B	B	B	A	B	B	B	B	B	B	A	B	A	A	A	A	B	A	B	B
29 *csu164	B	B	B	A	B	A	B	B	B	B	A	A	A	A	A	B	B	B	A	B
30 *csu25	B	B	B	B	B	A	A	A	B	A	A	A	A	B	A	B	B	B	A	-
31 *csu29	B	B	B	A	A	B	-	B	-	A	B	A	A	A	B	A	B	A	B	A
32 *csu31	B	B	A	A	B	A	B	B	A	B	B	A	A	A	A	A	B	B	B	A
33 *csu36	B	B	B	A	B	B	A	A	B	A	B	A	A	B	A	A	B	A	A	A
34 *csu39	B	B	A	B	B	A	A	A	A	B	A	B	B	B	B	B	B	A	B	A
35 *csu46	A	B	B	B	-	B	B	B	B	A	A	A	-	-	A	A	B	B	A	A
36 *csu50	A	B	A	A	A	A	B	B	B	A	B	A	A	-	A	A	A	A	A	A
37 *csu54	A	B	A	A	B	B	A	-	B	B	A	A	B	B	A	A	B	A	B	B
38 *csu59	A	B	A	B	B	B	A	B	B	A	B	B	B	A	B	B	B	A	B	A
39 *csu61	A	B	A	A	B	B	B	B	B	B	B	A	A	A	A	B	B	B	B	B
40 *csu92	A	B	B	B	A	A	B	A	A	-	B	B	B	A	A	B	B	A	B	A

Appendix E. Cont.

DNA Marker	RILs 1 I1	2 I2	3 I3	4 I4	5 I5	6 I6	7 I7	8 I8	9 I9	10 I10	11 I11	12 I12	13 I13	14 I14	15 I15	16 I16	17 I17	18 I18	19 I19	20 I21
41 *csu95a	A	B	A	B	B	B	A	B	A	A	A	A	A	B	A	B	B	A	A	B
42 *csu95b	B	B	A	A	A	B	B	B	B	B	A	A	A	B	A	A	B	B	B	B
43 *mag1f03	A	B	B	B	B	B	A	B	B	A	B	A	-	A	B	-	B	A	A	B
44 *npi105	B	B	B	B	A	A	A	B	B	B	A	A	B	B	B	B	B	B	B	B
45 *npi220	A	B	A	B	B	-	A	-	B	A	A	-	A	B	-	B	B	B	A	A
46 *npi232	A	B	B	B	A	A	A	A	B	A	A	A	A	A	B	A	B	B	A	A
47 *npi238	B	B	A	B	A	-	B	B	B	B	A	A	B	B	A	B	B	A	A	B
48 *npi239	B	B	B	B	A	B	-	B	-	B	B	A	B	B	B	B	B	A	B	A
49 *npi285	A	B	B	B	B	A	B	A	B	A	A	A	A	B	A	B	B	B	A	B
50 *npi287	A	B	B	B	A	A	A	B	B	A	A	A	A	A	B	A	B	B	A	A
51 *npi409	B	B	B	B	A	A	A	B	B	B	B	A	B	B	B	B	B	B	B	B
52 *npi451	B	B	A	A	B	A	A	B	A	A	B	A	A	B	B	B	B	A	A	A
53 *phi22	A	B	B	A	B	B	B	B	B	B	B	A	A	B	B	B	B	B	A	A
54 *phi93	A	B	B	A	B	A	A	B	A	A	B	B	B	A	B	B	B	B	A	A
55 *phi115	B	B	A	A	B	A	B	B	B	B	-	A	A	A	A	A	B	A	B	B
56 *php20581	A	B	A	B	-	A	A	A	A	-	-	A	-	-	-	-	A	-	-	A
57 *php4239	A	-	A	A	A	A	B	B	B	A	B	-	A	-	A	A	A	A	A	A
58 *umc102	B	B	A	-	B	-	A	B	B	A	-	B	A	B	B	B	-	B	A	A
59 *umc104a	A	B	A	A	B	A	A	A	B	A	B	A	B	B	B	A	B	B	B	A
60 *umc104b	A	B	A	A	B	A	B	B	B	B	A	A	B	A	A	B	B	A	A	B
61 *umc105	A	B	A	B	A	A	A	A	A	A	B	-	A	B	A	A	B	A	B	A
62 *umc107	A	B	B	A	B	A	B	B	B	B	A	A	B	A	A	B	B	A	A	B
63 *umc109	A	B	A	B	B	B	A	B	-	A	A	A	B	B	A	B	B	A	A	B
64 *umc110	B	B	-	A	B	B	-	-	-	B	B	-	B	A	A	A	B	B	A	B
65 *umc113	B	-	A	B	B	A	A	-	-	A	A	A	B	B	A	-	B	A	A	B
66 *umc114	B	A	A	A	A	B	B	B	B	B	A	A	A	A	B	B	A	B	B	A
67 *umc123	A	B	B	B	B	A	B	A	A	B	B	B	A	A	A	B	B	B	B	A
68 *umc124	B	B	A	A	B	A	A	A	A	B	A	A	B	B	A	A	B	A	A	B
69 *umc130	A	B	B	B	A	A	B	A	B	A	A	A	B	A	B	A	B	B	A	A
70 *umc132	B	B	A	A	A	B	A	A	A	B	A	A	A	A	A	A	B	B	A	A
71 *umc133	A	B	A	A	B	A	A	A	-	A	B	A	B	A	B	B	B	B	A	A
72 *umc135	B	B	A	B	A	A	A	B	A	A	A	B	A	B	B	B	B	A	A	A
73 *umc147	B	B	B	B	B	B	B	B	A	B	B	A	B	B	A	B	B	B	B	B
74 *umc148	A	B	A	B	B	B	A	B	-	A	A	A	B	B	A	B	B	A	A	B
75 *umc149	B	B	B	A	B	B	A	B	B	B	B	B	B	A	A	B	B	B	B	B
76 *umc14	B	B	-	A	B	B	A	-	B	B	B	A	B	B	A	A	B	A	A	B
77 *umc152	B	B	A	A	B	A	B	B	B	B	B	A	B	B	B	A	B	A	B	B
78 *umc156	A	B	B	A	A	B	A	A	B	B	B	A	B	B	A	A	B	A	A	A
79 *umc157a	A	B	A	A	A	B	B	-	A	B	-	A	-	A	B	B	B	A	A	B
80 *umc157b	A	B	A	A	A	B	B	B	A	B	A	-	A	A	B	B	B	A	-	B

Appendix E. Cont.

DNA Marker	RILs 1 I1	2 I2	3 I3	4 I4	5 I5	6 I6	7 I7	8 I8	9 I9	10 I10	11 I11	12 I12	13 I13	14 I14	15 I15	16 I16	17 I17	18 I18	19 I19	20 I21
81 *umc15	A	B	B	A	B	A	-	B	A	A	B	B	B	A	B	B	B	B	A	A
82 *umc164	A	B	A	B	A	B	B	A	B	B	B	A	B	A	B	A	B	B	B	B
83 *umc166	A	B	A	A	B	B	A	A	B	B	B	A	A	A	A	B	B	B	B	B
84 *umc167	A	-	A	A	B	A	-	B	A	-	B	A	A	-	-	-	-	B	B	B
85 *umc173	A	B	A	B	B	B	-	-	-	A	A	A	B	B	A	B	B	A	A	B
86 *umc17	B	B	A	-	A	A	A	A	A	A	B	B	A	B	A	B	B	A	B	A
87 *umc186	A	B	A	A	A	B	A	B	B	A	A	A	A	A	B	A	B	B	B	B
88 *umc18	-	B	A	-	B	B	A	-	B	A	-	A	B	B	B	B	B	B	A	B
89 *umc193	A	B	A	A	A	B	-	A	-	B	B	A	B	A	B	A	B	-	A	A
90 *umc199	-	-	-	-	-	-	-	-	-	-	-	-	-	B	-	-	-	A	A	A
91 *umc19	A	B	A	A	B	A	A	A	B	A	B	A	B	B	B	A	B	B	B	A
92 *umc26	A	B	B	A	A	B	B	-	A	B	B	B	B	B	-	B	B	A	B	A
93 *umc27	B	B	B	B	B	B	B	A	B	A	B	B	A	B	A	B	B	A	B	A
94 *umc28	A	B	B	A	A	B	B	A	A	A	B	B	B	B	B	B	B	A	A	A
95 *umc29	A	B	B	B	A	A	A	B	B	A	A	A	A	A	B	A	B	B	-	-
96 *umc30	B	B	A	A	B	B	B	A	A	B	B	A	A	A	A	A	B	B	B	A
97 *umc31a	B	B	B	B	A	A	B	A	A	B	B	B	B	A	A	B	B	A	B	A
98 *umc31b	-	B	B	B	A	A	B	A	A	-	B	B	B	A	A	B	B	A	B	A
99 *umc32	A	B	A	B	B	A	B	B	B	A	A	B	B	A	A	A	B	B	B	B
100 *umc36	B	A	A	A	B	B	B	A	B	B	A	B	A	A	A	A	B	B	B	A
101 *umc37	B	B	A	A	B	B	B	B	B	B	B	A	A	A	B	A	B	-	B	B
102 *umc38	B	B	A	A	A	B	A	B	A	B	A	A	B	B	A	B	B	B	A	B
103 *umc39	-	A	B	B	A	B	B	-	A	B	B	A	B	-	A	B	-	-	B	B
104 *umc45	B	B	B	A	A	B	A	B	-	B	-	A	A	A	A	A	B	A	A	A
105 *umc48	B	B	A	A	B	B	B	A	A	B	B	A	A	A	A	A	B	B	-	A
106 *umc50	B	B	A	B	B	A	A	B	A	-	A	B	B	B	B	B	B	B	A	B
107 *umc51	A	B	B	B	A	B	B	A	A	B	A	B	B	B	A	B	B	A	B	A
108 *umc55	A	B	A	A	B	B	A	A	B	A	A	A	B	B	B	A	B	A	B	B
109 *umc59	B	B	A	A	A	B	B	A	B	A	A	B	B	A	A	-	B	A	A	B
110 *umc5	A	B	-	A	B	B	A	-	B	-	-	A	A	B	A	A	B	A	A	B
111 *umc65	B	B	A	A	B	B	B	B	B	B	B	A	A	A	A	A	B	A	B	B
112 *umc66	A	B	A	A	B	A	A	A	B	A	B	A	B	B	B	A	B	B	B	A
113 *umc67	A	B	B	A	-	A	-	-	B	B	A	A	B	A	A	B	B	A	A	B
114 *umc72	B	A	B	B	B	B	B	B	A	B	B	A	A	B	A	B	A	B	A	B
115 *umc76	A	-	A	B	B	B	A	B	-	B	B	B	-	A	B	-	B	-	B	A
116 *umc80	B	A	A	B	A	B	A	B	B	A	A	A	B	A	A	A	A	B	B	B
117 *umc89	B	B	A	A	B	-	B	A	A	B	B	B	A	A	A	A	B	B	B	A
118 *umc92	A	B	A	B	B	B	A	B	A	B	A	B	B	B	B	B	B	B	A	A
119 *umc96	-	B	B	A	B	-	A	A	B	A	B	A	A	-	A	A	B	A	-	B
120 *umc97	A	A	B	B	A	A	B	B	B	A	A	B	B	B	B	B	A	A	A	B

Appendix E. Cont.

DNA Marker	RILs																			
	21 I22	22 I24	23 I25	24 I26	25 I27	26 I28	27 I29	28 I30	29 I31	30 I32	31 I33	32 I34	33 I36	34 I38	35 I39	36 I40	37 I41	38 I42	39 I44	40 I45
1 *asg30	A	A	B	B	B	B	B	B	A	B	B	B	A	B	B	B	B	B	A	B
2 *asg62	B	A	B	B	B	B	A	A	B	B	-	B	B	B	B	A	B	B	A	A
3 *asg75	A	A	B	B	B	B	B	B	A	B	B	B	A	B	B	B	B	B	B	A
4 *bnl10.24	A	A	A	B	A	A	B	-	A	B	B	B	B	B	B	A	B	B	A	-
5 *bnl12.09	A	B	A	A	-	A	A	-	B	A	A	B	B	B	B	B	A	A	B	B
6 *bnl12.30	A	-	B	B	B	B	B	B	A	B	B	A	A	A	A	A	A	A	B	A
7 *bnl12.30b	B	-	A	B	B	B	B	B	B	A	B	B	B	B	B	B	A	A	B	A
8 *bnl13.05	B	A	A	B	A	A	A	B	B	A	A	B	B	A	B	B	B	B	B	A
9 *bnl16.06	A	A	B	B	A	A	B	A	B	B	B	A	B	B	A	B	B	B	A	A
10 *bnl3.04	B	A	B	B	B	A	-	A	A	A	A	A	-	A	B	A	A	A	-	A
11 *bnl4.06	B	B	B	B	B	A	A	B	B	A	A	A	B	A	B	A	B	B	A	A
12 *bnl5.40	B	-	B	A	B	B	A	B	B	A	B	B	B	A	A	A	B	B	B	A
13 *bnl5.46	B	A	A	A	B	B	A	-	B	B	B	B	A	A	A	B	B	B	A	B
14 *bnl5.62	B	A	B	B	A	B	-	A	A	B	A	A	B	B	B	B	A	A	B	-
15 *bnl6.25	A	-	A	A	A	-	-	A	A	-	A	A	-	-	A	A	A	A	A	-
16 *bnl7.49	A	B	A	A	-	-	A	A	A	-	B	B	A	A	B	A	A	A	A	A
17 *bnl7.71	B	A	B	A	B	A	A	B	B	A	A	A	B	A	B	A	B	B	B	A
18 *bnl8.01	A	A	A	B	A	A	B	A	A	B	B	B	B	B	B	A	B	B	A	A
19 *bnl8.17	A	A	A	B	A	B	A	B	A	B	B	B	B	B	A	A	-	A	A	B
20 *bnl8.39	B	A	A	B	A	B	B	B	B	-	A	B	-	B	A	B	B	B	B	A
21 *bnl8.45	B	B	A	B	B	B	B	B	A	A	B	A	A	B	A	A	B	B	A	A
22 *csu110	A	A	B	A	A	A	A	A	B	A	A	B	A	A	B	A	B	B	A	B
23 *csu11	B	A	B	B	A	A	-	B	A	A	A	B	A	B	A	A	B	B	A	A
24 *csu12	A	-	B	B	A	A	B	B	A	B	A	A	A	B	B	A	A	A	B	B
25 *csu136	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
26 *csu13	A	-	A	B	B	B	B	-	-	-	-	A	A	B	A	B	A	A	B	B
27 *csu145	B	A	A	A	-	-	A	A	B	A	-	A	B	B	B	A	A	-	B	A
28 *csu146	B	B	B	B	B	B	B	B	B	B	B	B	A	A	B	B	B	B	B	B
29 *csu164	B	A	B	A	B	B	A	A	B	A	B	-	A	B	B	B	B	B	A	B
30 *csu25	B	A	B	B	B	A	A	B	B	A	A	A	B	A	B	A	-	A	A	B
31 *csu29	B	A	A	B	A	B	-	B	B	A	A	B	B	A	-	B	-	-	A	B
32 *csu31	A	A	A	A	A	A	A	A	A	B	A	B	A	A	A	B	B	B	A	B
33 *csu36	A	-	B	B	B	B	B	A	A	B	B	A	A	A	A	A	A	A	B	B
34 *csu39	A	A	B	B	B	A	B	A	A	B	B	A	B	B	B	A	B	B	A	A
35 *csu46	B	A	A	B	A	A	A	B	B	A	A	A	A	B	B	A	B	B	B	B
36 *csu50	A	-	-	B	B	A	B	A	A	A	A	B	B	A	B	B	A	B	B	B
37 *csu54	A	B	B	A	A	A	A	A	-	A	A	B	A	B	A	-	-	B	B	A
38 *csu59	A	-	B	B	A	B	A	B	B	B	B	A	B	B	A	B	A	A	B	A
39 *csu61	B	A	B	-	B	B	A	B	B	B	B	B	A	B	A	A	B	B	A	A
40 *csu92	A	-	-	B	B	B	B	B	A	A	B	B	-	-	A	B	A	A	A	A

Appendix E. Cont.

	RILs																				
DNA Marker	20 I21	21 I22	22 I24	23 I25	24 I26	25 I27	26 I28	27 I29	28 I30	29 I31	30 I32	31 I33	32 I34	33 I36	34 I38	35 I39	36 I40	37 I41	38 I42	39 I44	40 I45
41 *csu95a	B	A	A	A	B	B	A	A	A	B	A	B	A	A	B	A	A	A	A	A	B
42 *csu95b	B	B	A	B	B	A	A	B	-	-	B	A	A	A	A	-	A	B	B	A	A
43 *mag1f03	B	B	A	A	-	B	B	A	B	B	B	B	B	A	A	B	B	B	B	A	A
44 *npi105	B	A	A	A	A	B	A	B	A	A	B	A	A	B	B	A	A	A	A	B	A
45 *npi220	A	B	-	A	B	A	B	B	B	B	B	A	B	A	B	A	B	B	B	A	B
46 *npi232	A	B	B	B	B	A	A	B	B	B	A	A	B	A	B	B	A	B	B	B	B
47 *npi238	B	B	B	B	B	A	A	A	B	A	A	A	A	B	B	A	A	B	B	B	B
48 *npi239	A	A	-	A	B	B	B	B	A	A	A	A	B	B	B	-	B	A	A	B	B
49 *npi285	B	B	B	A	B	A	A	A	B	B	B	A	A	A	A	A	A	A	A	B	B
50 *npi287	A	B	A	B	A	A	A	A	B	B	A	B	A	B	A	A	A	B	B	B	A
51 *npi409	B	A	A	A	A	B	A	B	A	A	B	A	A	B	B	A	A	-	A	-	B
52 *npi451	A	B	A	A	B	A	B	B	B	A	A	A	-	A	B	A	B	B	B	A	A
53 *phi22	A	-	-	-	-	A	A	A	B	B	B	A	A	B	B	A	A	A	A	B	A
54 *phi93	A	B	A	B	B	B	B	B	A	A	B	A	A	B	A	A	B	B	B	B	A
55 *phi115	B	A	A	A	B	A	A	B	A	A	A	A	B	A	A	-	B	A	A	A	B
56 *php20581	A	-	A	-	A	A	B	B	B	A	-	A	-	-	-	-	B	A	A	B	-
57 *php4239	A	B	B	-	A	B	A	B	A	A	A	A	B	B	B	B	B	A	-	B	B
58 *umc102	A	A	B	A	B	A	B	-	B	A	B	A	B	A	B	A	B	-	A	B	A
59 *umc104a	A	B	A	A	B	B	B	B	B	A	A	B	B	A	B	B	A	B	B	A	-
60 *umc104b	B	B	A	B	A	B	B	A	B	A	A	B	A	B	B	B	B	B	B	A	B
61 *umc105	A	A	A	B	B	B	B	B	B	A	B	B	B	B	A	B	A	A	A	A	A
62 *umc107	B	B	A	B	A	B	B	A	A	A	A	B	A	B	B	B	B	B	B	A	B
63 *umc109	B	A	A	A	B	B	A	A	B	B	A	A	A	A	B	A	A	A	A	A	B
64 *umc110	B	B	A	B	-	A	A	B	A	A	B	A	B	B	B	A	-	B	B	B	B
65 *umc113	B	A	-	A	-	B	A	A	-	-	-	-	A	A	B	A	A	A	A	A	B
66 *umc114	A	B	B	B	B	B	B	A	B	B	B	B	A	A	A	B	B	B	B	B	A
67 *umc123	A	B	B	B	B	-	B	B	B	B	A	B	B	B	A	B	B	B	B	B	B
68 *umc124	B	A	B	A	A	A	A	A	A	A	A	A	B	A	B	A	-	A	A	A	B
69 *umc130	A	B	A	A	B	A	A	-	B	B	B	A	A	A	B	B	A	B	B	B	B
70 *umc132	A	A	A	A	A	A	A	A	A	B	A	A	A	A	B	A	B	A	A	A	A
71 *umc133	A	B	A	A	B	B	B	B	B	A	A	B	A	A	B	A	A	-	B	A	A
72 *umc135	A	A	A	A	B	A	A	B	A	A	B	B	B	B	B	B	A	B	B	A	A
73 *umc147	B	A	A	A	A	B	A	-	A	A	B	B	B	B	B	A	B	A	A	B	B
74 *umc148	B	A	A	A	B	B	A	A	B	B	A	A	A	A	B	A	A	-	A	A	B
75 *umc149	B	B	A	B	B	A	A	B	A	A	B	A	B	A	B	A	B	B	B	B	B
76 *umc14	B	A	A	B	A	A	A	A	A	B	A	A	B	-	B	B	A	-	B	B	A
77 *umc152	B	A	B	A	B	A	A	A	A	A	B	A	B	B	B	A	B	A	A	-	B
78 *umc156	A	-	B	A	B	B	A	-	A	A	A	B	A	B	B	A	-	B	B	A	A
79 *umc157a	B	B	B	B	B	A	A	B	A	B	A	B	B	B	B	B	B	A	A	B	A
80 *umc157b	B	B	B	B	B	A	A	B	A	B	-	B	B	B	B	-	B	A	A	B	A

Appendix E. Cont.

DNA Marker	RILs																			
	21 I22	22 I24	23 I25	24 I26	25 I27	26 I28	27 I29	28 I30	29 I31	30 I32	31 I33	32 I34	33 I36	34 I38	35 I39	36 I40	37 I41	38 I42	39 I44	40 I45
81 *umc15	B	A	B	B	B	A	-	B	A	A	A	A	A	B	A	A	B	B	A	A
82 *umc164	-	B	B	B	A	B	B	A	B	B	A	A	B	B	B	B	A	A	B	A
83 *umc166	B	-	B	B	B	B	A	B	B	B	B	B	A	B	A	A	B	B	A	A
84 *umc167	B	A	B	B	B	B	A	-	A	A	B	B	A	-	A	B	B	B	-	A
85 *umc173	A	A	A	B	B	A	A	B	B	A	A	-	A	B	A	A	A	A	B	A
86 *umc17	A	A	B	B	B	A	B	A	A	B	B	B	B	B	B	A	B	B	A	A
87 *umc186	A	A	A	B	A	A	B	A	B	B	A	B	A	A	A	B	A	B	A	B
88 *umc18	A	-	B	A	A	A	A	A	B	A	A	B	A	A	B	A	B	B	A	B
89 *umc193	A	B	A	B	A	B	B	-	B	A	B	A	A	A	-	A	-	-	A	B
90 *umc199	-	-	-	-	B	A	-	A	A	-	A	A	B	A	A	A	B	A	A	A
91 *umc19	B	A	A	B	B	B	B	B	A	A	-	B	A	B	B	A	B	B	A	A
92 *umc26	B	A	B	B	B	B	A	B	B	A	A	B	B	A	A	B	B	B	A	A
93 *umc27	B	B	B	A	B	A	A	B	A	A	A	A	B	B	B	A	B	B	A	A
94 *umc28	B	B	B	A	B	B	A	B	B	A	B	B	A	A	A	A	B	B	B	A
95 *umc29	B	A	B	B	A	A	A	B	B	A	B	A	B	B	B	A	B	B	B	B
96 *umc30	A	A	A	A	A	A	A	A	A	B	A	B	A	A	A	B	A	B	A	B
97 *umc31a	A	B	A	B	B	B	-	B	A	A	B	B	B	B	A	B	A	A	A	A
98 *umc31b	A	A	A	B	B	B	B	B	A	A	B	B	-	-	A	B	A	A	A	A
99 *umc32	B	A	A	B	A	A	B	B	A	B	A	B	A	A	B	B	A	A	A	A
100 *umc36	A	A	A	B	-	A	A	B	A	B	A	B	A	A	B	B	B	B	A	B
101 *umc37	A	A	A	B	A	A	A	A	A	B	-	B	A	-	A	B	B	B	A	B
102 *umc38	B	B	A	B	A	A	-	B	A	B	A	A	B	A	B	A	B	B	A	A
103 *umc39	-	-	-	-	B	-	-	-	B	-	A	B	B	-	-	B	B	B	-	-
104 *umc45	A	A	B	B	A	A	B	A	B	-	B	A	B	B	A	B	B	B	B	B
105 *umc48	A	A	A	A	A	A	A	A	A	B	A	B	A	A	A	B	B	B	A	B
106 *umc50	B	A	A	B	A	B	A	A	B	B	A	B	A	B	A	B	-	B	B	A
107 *umc51	B	A	B	B	B	B	A	B	B	A	B	B	B	A	A	A	B	B	A	A
108 *umc55	A	A	B	A	B	A	A	A	B	A	A	A	A	B	A	A	B	B	A	B
109 *umc59	A	A	B	A	B	A	B	B	B	A	A	A	A	A	B	A	B	B	B	B
110 *umc5	A	-	B	A	A	A	A	A	B	A	A	A	B	B	-	A	B	B	A	B
111 *umc65	A	A	B	B	B	A	B	B	B	B	A	A	A	B	B	A	B	B	A	A
112 *umc66	B	A	A	B	B	B	B	A	A	A	-	A	A	B	B	-	-	-	A	A
113 *umc67	B	A	B	A	B	B	A	A	A	A	B	A	B	A	B	B	A	-	-	B
114 *umc72	A	A	A	A	A	A	-	B	A	B	A	A	B	B	A	A	B	B	A	B
115 *umc76	A	-	A	B	A	B	-	B	B	B	A	B	-	-	B	B	A	A	A	-
116 *umc80	B	-	A	B	-	-	B	-	B	B	-	B	A	B	A	B	B	B	B	B
117 *umc89	A	A	B	B	B	B	B	B	B	A	B	B	A	A	A	B	B	B	A	B
118 *umc92	B	A	A	B	A	B	B	B	B	B	A	B	A	B	A	B	B	B	B	A
119 *umc96	A	-	-	B	B	A	B	A	A	B	B	A	A	-	A	A	A	A	B	B
120 *umc97	B	B	B	B	B	B	B	B	B	A	B	A	B	B	B	A	A	A	B	A

Appendix E. Cont.

	RILs																			
DNA Marker	41 I46	42 I47	43 I48	44 I49	45 I50	46 I51	47 I52	48 I53	49 I54	50 I55	51 I56	52 I57	53 I58	54 I59	55 I60	56 I62	57 I63	58 I64	59 I66	60 I67
1 *asg30	B	A	-	B	A	A	A	A	A	A	-	A	B	B	B	B	-	B	-	A
2 *asg62	B	A	B	B	B	-	A	B	A	A	A	-	A	A	B	B	A	B	-	B
3 *asg75	B	-	-	-	A	-	A	A	A	A	-	A	B	B	B	B	B	-	-	A
4 *bnl10.24	B	A	A	B	B	B	B	-	B	A	-	A	A	A	B	B	-	B	A	A
5 *bnl12.09	B	A	A	B	B	-	A	A	B	A	A	B	A	A	B	B	A	A	A	A
6 *bnl12.30	A	B	A	A	B	A	B	B	B	B	A	A	A	A	B	B	A	B	A	A
7 *bnl12.30b	B	B	A	B	B	B	A	B	B	B	B	B	B	B	B	B	A	A	A	B
8 *bnl13.05	B	A	B	A	A	B	B	B	B	B	A	A	A	A	A	A	B	B	-	A
9 *bnl16.06	A	A	A	B	A	B	B	-	A	A	A	-	A	A	A	A	A	A	-	A
10 *bnl3.04	-	A	A	A	B	B	B	A	B	B	A	A	A	B	A	A	A	A	B	B
11 *bnl4.06	B	A	A	A	B	-	B	B	B	A	A	A	A	B	B	A	B	B	B	A
12 *bnl5.40	B	B	B	A	B	B	B	B	B	B	B	A	A	A	B	A	-	B	B	B
13 *bnl5.46	B	A	B	A	B	B	A	-	A	-	A	B	B	B	B	A	B	A	B	B
14 *bnl5.62	A	A	A	B	A	B	B	B	B	B	B	B	B	A	B	A	B	B	B	-
15 *bnl6.25	A	A	A	-	B	B	A	A	-	B	B	B	A	A	-	B	-	A	B	A
16 *bnl7.49	-	-	B	-	-	-	A	-	-	-	A	A	A	B	B	A	-	A	A	B
17 *bnl7.71	B	B	A	A	B	-	B	B	-	A	A	A	A	B	B	A	B	B	A	B
18 *bnl8.01	B	A	A	B	B	A	B	A	B	A	A	A	A	A	B	B	B	A	-	A
19 *bnl8.17	A	B	B	B	A	B	B	A	-	A	-	A	A	B	A	A	A	B	A	B
20 *bnl8.39	A	A	B	B	B	B	B	A	A	A	A	A	A	B	A	A	A	B	A	B
21 *bnl8.45	A	B	B	A	A	A	A	A	A	B	A	A	A	B	B	B	A	A	B	B
22 *csu110	-	A	B	A	B	B	A	B	B	-	-	-	-	-	-	-	-	-	-	-
23 *csu11	B	A	B	B	B	A	A	B	B	-	A	A	A	B	B	B	A	A	B	B
24 *csu12	A	-	B	A	A	-	B	A	A	B	A	A	B	B	B	B	B	A	-	B
25 *csu136	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A
26 *csu13	B	A	A	B	B	B	A	-	B	A	A	B	B	A	A	A	A	A	B	A
27 *csu145	A	B	B	B	A	A	A	-	A	B	-	A	B	B	B	A	B	A	-	A
28 *csu146	B	A	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B	B	A	-	B
29 *csu164	A	A	A	A	A	A	A	B	B	A	B	B	A	A	B	B	-	A	-	B
30 *csu25	A	A	B	A	B	B	B	A	B	B	B	A	A	B	A	A	B	B	A	B
31 *csu29	B	A	B	A	A	B	-	-	B	A	-	A	A	-	A	B	B	A	A	A
32 *csu31	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	B	B	B	A
33 *csu36	A	B	A	-	B	A	B	B	B	B	A	A	A	A	B	B	A	B	-	A
34 *csu39	B	A	A	B	B	-	B	A	A	B	B	A	A	A	B	B	A	B	A	A
35 *csu46	A	A	B	B	A	-	A	A	A	B	A	A	A	-	B	A	A	A	A	A
36 *csu50	B	-	A	A	B	B	A	A	B	A	A	B	A	A	A	A	B	A	B	A
37 *csu54	B	A	B	B	B	B	A	B	B	A	B	B	A	A	B	B	-	A	-	A
38 *csu59	B	B	A	B	A	B	B	A	B	B	A	A	A	B	B	A	B	-	-	B
39 *csu61	B	A	B	B	A	-	B	A	A	-	-	B	A	B	B	B	B	B	A	-
40 *csu92	A	B	A	A	B	A	A	A	B	A	B	A	A	A	B	B	B	A	A	A

Appendix E. Cont.

DNA Marker	RILs 41 I46	42 I47	43 I48	44 I49	45 I50	46 I51	47 I52	48 I53	49 I54	50 I55	51 I56	52 I57	53 I58	54 I59	55 I60	56 I62	57 I63	58 I64	59 I66	60 I67
41 *csu95a	A	B	B	B	A	A	B	A	B	A	B	B	B	A	A	B	B	B	A	B
42 *csu95b	-	A	A	A	A	A	A	B	-	B	B	-	A	A	A	B	A	A	-	B
43 *mag1f03	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	A	B	B	B	B
44 *npi105	A	B	A	B	B	B	A	A	B	B	A	B	A	A	B	B	A	B	B	A
45 *npi220	A	A	B	B	B	B	B	A	B	-	A	A	A	-	-	A	B	A	-	A
46 *npi232	A	B	B	B	A	A	A	B	A	B	A	A	A	A	B	B	A	A	A	B
47 *npi238	A	A	B	B	-	-	B	B	B	B	B	B	A	A	A	A	B	B	B	B
48 *npi239	B	A	B	B	B	A	A	A	A	B	B	B	A	A	B	B	A	B	-	A
49 *npi285	A	A	A	A	B	A	B	B	B	B	A	A	A	B	A	A	B	B	A	B
50 *npi287	B	A	B	A	A	-	A	B	A	B	A	A	A	A	B	A	A	A	B	B
51 *npi409	A	A	A	B	B	-	A	A	B	B	A	B	A	A	B	B	B	A	B	A
52 *npi451	B	A	A	B	B	-	B	-	B	A	A	A	B	B	B	A	B	B	A	B
53 *phi22	A	B	B	B	B	B	B	A	B	A	B	B	A	A	A	A	B	B	B	B
54 *phi93	A	A	B	B	A	B	A	B	B	B	A	A	A	B	B	B	A	A	A	B
55 *phi115	A	A	B	A	A	B	A	B	A	A	A	A	A	A	A	B	-	A	-	-
56 *php20581	B	-	A	-	B	-	A	A	-	-	-	-	A	-	-	-	A	A	-	A
57 *php4239	A	-	A	A	B	B	A	A	B	A	A	B	A	A	A	B	B	A	B	A
58 *umc102	A	A	B	B	B	-	B	A	B	A	A	A	A	-	A	A	A	B	B	A
59 *umc104a	B	B	B	A	-	-	A	A	A	B	A	A	A	A	B	A	A	A	A	B
60 *umc104b	A	A	A	B	A	A	A	B	B	A	B	B	A	A	B	B	B	A	B	B
61 *umc105	B	A	A	A	B	B	B	B	A	B	A	A	A	B	B	A	A	B	A	B
62 *umc107	A	A	A	B	A	A	A	B	B	A	B	B	A	A	B	B	B	A	B	B
63 *umc109	A	B	B	B	A	-	B	A	A	B	B	B	B	A	A	-	B	B	B	-
64 *umc110	A	A	A	B	A	-	B	B	A	B	-	B	B	B	B	B	A	A	A	-
65 *umc113	A	-	B	B	-	A	-	A	B	-	-	-	B	A	A	-	-	-	-	-
66 *umc114	B	A	A	A	A	A	B	B	B	B	B	B	A	A	B	B	B	A	B	A
67 *umc123	A	A	B	A	B	A	A	B	B	A	B	B	A	A	B	A	A	A	B	B
68 *umc124	B	A	A	B	B	B	A	A	B	A	A	A	A	A	B	B	A	A	A	A
69 *umc130	A	A	B	B	B	A	A	A	B	B	A	A	A	B	B	A	A	A	A	B
70 *umc132	A	A	A	A	A	A	A	A	A	A	B	B	A	B	A	A	A	A	A	A
71 *umc133	A	B	B	A	A	A	A	A	B	B	A	A	A	B	B	B	A	A	B	B
72 *umc135	B	A	A	B	B	A	B	A	B	A	B	A	A	A	B	B	A	B	A	A
73 *umc147	B	A	A	B	B	B	B	A	A	B	B	B	A	B	-	B	B	A	B	A
74 *umc148	A	B	B	B	A	B	B	-	A	B	-	B	B	A	A	B	B	B	B	-
75 *umc149	A	A	A	B	A	A	B	B	A	B	A	B	B	B	B	B	A	A	A	B
76 *umc14	B	A	B	B	B	B	A	B	B	A	B	B	A	-	B	B	A	A	A	A
77 *umc152	-	A	B	-	-	B	A	-	A	A	A	A	B	A	B	B	A	A	-	A
78 *umc156	B	B	A	B	B	A	A	A	B	B	-	A	B	B	A	A	B	A	A	A
79 *umc157a	B	B	B	B	A	B	B	B	A	A	A	A	B	A	B	B	-	B	B	A
80 *umc157b	B	B	B	B	A	B	B	-	A	A	A	A	B	A	B	B	B	B	B	A

Appendix E. Cont.

	RILs																			
DNA Marker	41 I46	42 I47	43 I48	44 I49	45 I50	46 I51	47 I52	48 I53	49 I54	50 I55	51 I56	52 I57	53 I58	54 I59	55 I60	56 I62	57 I63	58 I64	59 I66	60 I67
81 *umc15	A	B	B	A	B	A	B	B	A	B	A	A	A	B	B	B	A	A	A	B
82 *umc164	A	A	B	B	B	A	A	B	B	B	B	A	B	A	B	A	A	B	A	B
83 *umc166	B	A	B	B	A	A	B	A	A	A	B	B	A	B	B	B	B	B	-	A
84 *umc167	B	B	B	-	A	A	-	-	A	A	-	-	A	-	-	-	-	B	A	A
85 *umc173	A	B	B	B	-	B	B	A	A	B	-	B	B	A	A	B	B	B	-	B
86 *umc17	B	A	A	A	B	A	B	B	A	B	A	A	A	-	B	A	B	A	A	B
87 *umc186	A	A	A	A	A	-	A	A	B	A	A	A	B	B	A	A	A	A	A	A
88 *umc18	B	A	B	A	B	B	-	B	B	-	B	B	A	A	B	B	-	-	-	-
89 *umc193	A	B	-	A	A	A	A	-	B	B	B	A	A	B	A	A	B	A	-	-
90 *umc199	A	A	A	A	-	A	B	-	-	-	A	A	A	A	-	A	A	B	A	A
91 *umc19	B	B	B	A	B	-	A	A	A	B	A	A	A	A	B	A	A	A	A	B
92 *umc26	B	B	B	A	B	B	B	B	-	A	B	B	A	A	B	A	A	A	B	B
93 *umc27	A	A	A	B	B	A	B	B	A	A	A	A	A	B	B	A	B	B	B	B
94 *umc28	B	B	A	A	B	A	B	B	B	B	A	A	A	A	B	A	B	B	-	B
95 *umc29	A	A	B	B	A	B	A	-	A	B	A	A	A	A	B	A	A	A	B	B
96 *umc30	A	A	A	A	A	A	B	A	B	A	A	A	A	A	A	B	B	B	A	B
97 *umc31a	A	B	A	B	B	A	A	A	B	-	-	A	A	A	B	B	B	A	A	A
98 *umc31b	A	B	A	-	B	A	A	-	B	A	B	A	A	A	B	B	B	A	-	A
99 *umc32	B	A	B	A	B	B	A	A	B	B	B	B	A	B	A	B	A	B	-	B
100 *umc36	A	A	A	A	-	A	B	-	A	A	B	A	B	A	B	B	A	B	A	B
101 *umc37	A	A	B	-	A	B	B	B	A	A	A	A	B	A	B	B	A	B	A	A
102 *umc38	A	-	B	B	A	A	B	A	A	B	B	B	A	A	B	B	B	A	B	B
103 *umc39	B	-	A	-	B	A	-	B	B	-	B	B	A	-	B	A	B	B	B	-
104 *umc45	A	A	A	B	A	A	A	A	A	A	A	A	A	A	B	A	B	A	A	A
105 *umc48	A	A	A	A	A	A	B	-	B	A	A	A	A	A	A	B	B	B	-	A
106 *umc50	A	A	B	B	B	A	B	A	A	A	A	B	-	B	A	-	A	B	A	B
107 *umc51	B	B	B	A	B	B	B	-	B	B	B	A	A	A	B	A	B	A	B	B
108 *umc55	B	A	B	B	B	B	A	B	B	A	B	B	A	-	B	B	A	A	-	A
109 *umc59	A	A	B	B	A	-	B	B	A	B	-	B	A	B	A	A	B	A	A	A
110 *umc5	B	A	B	B	B	A	A	B	A	A	A	B	A	B	-	B	A	A	-	A
111 *umc65	A	A	A	B	A	A	B	B	A	B	A	B	A	A	A	B	B	A	B	B
112 *umc66	B	B	B	-	B	-	A	A	A	B	A	A	A	A	B	A	A	A	A	B
113 *umc67	A	A	A	B	A	A	A	B	B	A	B	B	A	A	B	B	B	A	B	B
114 *umc72	A	A	A	B	B	B	B	A	A	B	B	B	A	B	B	A	B	B	B	B
115 *umc76	B	-	A	-	-	B	-	A	B	A	A	A	B	B	B	B	-	-	A	A
116 *umc80	B	B	A	B	B	B	A	A	-	A	B	B	B	B	-	B	A	B	-	A
117 *umc89	A	A	B	A	B	A	A	B	A	A	A	A	-	A	A	B	B	-	B	A
118 *umc92	A	A	B	B	B	-	B	A	A	A	A	A	A	B	A	A	A	B	A	B
119 *umc96	A	B	-	-	B	A	B	B	A	B	A	-	-	A	B	B	A	-	A	A
120 *umc97	B	B	B	B	B	B	A	-	A	B	B	B	B	B	B	A	A	A	-	B

Appendix E. Cont.

DNA Marker	RILs 61 I68	62 I69	63 I70	64 I71	65 I72	66 I73	67 I74	68 I75	69 I76	70 I77	71 I78	72 I79	73 I80	74 I81	75 I83	76 I84	77 I85	78 I86	79 I87	80 I89
1 *asg30	A	A	A	B	A	A	A	B	B	A	A	B	B	B	-	A	B	A	B	B
2 *asg62	B	B	A	A	A	B	B	A	B	A	A	B	B	B	B	A	A	A	B	B
3 *asg75	A	A	A	B	A	A	A	B	B	A	A	B	B	B	B	A	B	A	B	B
4 *bnl10.24	-	-	B	B	A	B	-	B	A	A	B	A	B	A	A	A	B	A	A	A
5 *bnl12.09	B	B	A	-	-	A	A	B	A	A	B	B	B	A	B	-	A	B	B	B
6 *bnl12.30	B	B	A	A	A	B	A	B	A	A	A	A	B	A	A	-	A	A	B	A
7 *bnl12.30b	B	B	B	A	B	B	B	A	B	B	B	B	B	B	B	A	B	A	A	B
8 *bnl13.05	B	B	-	A	A	B	A	A	A	B	A	A	B	B	A	-	A	A	A	B
9 *bnl16.06	-	-	-	B	B	B	A	B	A	A	A	A	B	B	B	B	B	A	B	B
10 *bnl3.04	-	A	A	A	A	B	A	B	A	A	A	-	-	A	-	A	A	A	B	B
11 *bnl4.06	B	A	A	B	B	B	B	-	A	A	A	B	B	B	A	B	A	A	B	B
12 *bnl5.40	A	A	A	B	B	B	A	B	A	A	A	A	B	-	B	A	B	A	B	-
13 *bnl5.46	B	-	B	B	A	-	-	A	B	A	-	A	B	A	B	B	A	A	B	B
14 *bnl5.62	A	A	A	A	B	A	B	A	B	A	A	B	A	B	B	-	B	A	A	B
15 *bnl6.25	B	A	A	A	A	-	A	-	-	-	-	B	-	B	A	B	-	A	-	A
16 *bnl7.49	B	A	-	A	A	A	A	A	-	-	A	A	-	A	B	-	A	-	-	A
17 *bnl7.71	B	A	A	B	B	B	B	A	A	A	A	B	B	B	A	B	A	A	B	A
18 *bnl8.01	A	A	-	B	A	B	A	B	A	A	B	A	B	A	A	A	B	A	A	B
19 *bnl8.17	B	B	B	B	B	B	B	B	B	B	B	B	A	A	B	-	A	B	A	A
20 *bnl8.39	B	B	A	B	A	B	A	A	B	A	A	B	B	A	A	A	A	A	A	A
21 *bnl8.45	B	-	B	B	B	A	B	A	B	B	B	A	B	A	A	-	B	A	B	A
22 *csu110	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	A	B	B	B
23 *csu11	A	A	B	B	B	B	B	A	B	A	A	-	B	B	-	A	A	A	A	A
24 *csu12	A	A	B	B	A	B	B	B	B	B	A	B	B	B	B	A	B	A	B	A
25 *csu136	A	A	B	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	B	A
26 *csu13	A	A	A	B	A	A	B	B	A	A	B	B	B	B	-	A	B	B	A	A
27 *csu145	-	-	-	B	A	-	B	A	A	A	A	A	B	B	-	-	A	-	-	-
28 *csu146	B	A	B	A	B	B	A	B	A	B	B	A	B	A	B	B	B	B	B	B
29 *csu164	A	B	-	A	A	-	B	B	A	A	-	-	A	A	B	B	-	B	A	A
30 *csu25	B	A	A	A	A	A	A	B	A	A	A	B	B	A	A	B	A	-	B	B
31 *csu29	B	B	B	A	A	-	A	A	A	B	A	A	B	-	A	-	B	A	-	B
32 *csu31	A	A	B	B	A	A	A	B	A	B	A	A	A	B	A	-	A	B	B	A
33 *csu36	B	B	A	A	A	-	B	B	A	A	A	A	B	A	A	-	A	A	B	B
34 *csu39	B	B	A	B	A	B	A	B	-	B	B	A	B	A	A	-	-	-	-	-
35 *csu46	B	A	B	B	B	-	A	B	-	A	A	B	B	A	A	B	A	A	A	A
36 *csu50	A	-	-	B	A	B	A	B	B	-	B	B	B	B	B	A	A	B	A	A
37 *csu54	A	B	A	A	B	A	A	B	B	B	B	A	B	A	A	-	-	B	B	B
38 *csu59	B	B	B	B	B	B	B	B	B	B	B	A	B	A	B	A	A	B	A	B
39 *csu61	A	B	A	B	A	B	B	B	B	A	A	B	B	A	B	B	B	A	A	-
40 *csu92	A	A	A	B	A	A	A	-	B	A	A	B	B	B	A	-	-	A	A	A

Appendix E. Cont.

DNA Marker	RILs 61 I68	62 I69	63 I70	64 I71	65 I72	66 I73	67 I74	68 I75	69 I76	70 I77	71 I78	72 I79	73 I80	74 I81	75 I83	76 I84	77 I85	78 I86	79 I87	80 I89
41 *csu95a	B	B	A	A	B	B	A	B	B	A	A	B	B	B	A	A	A	A	B	A
42 *csu95b	A	B	B	A	B	A	A	B	B	B	A	A	A	A	B	A	B	A	B	B
43 *mag1f03	B	A	B	B	-	B	B	A	B	A	B	-	B	B	A	B	A	B	B	B
44 *npi105	B	A	A	A	A	A	A	B	B	A	B	A	B	B	A	B	B	A	B	A
45 *npi220	B	B	-	B	A	-	A	A	B	A	A	B	A	A	A	-	A	A	B	A
46 *npi232	B	B	B	B	A	A	A	A	A	A	A	B	B	A	A	A	B	A	A	A
47 *npi238	A	-	A	A	A	B	B	A	A	A	A	A	B	A	B	B	A	A	A	A
48 *npi239	B	B	-	A	B	A	-	B	A	B	B	B	B	A	B	A	B	B	A	B
49 *npi285	B	A	B	B	A	B	A	B	A	A	A	B	B	A	A	-	A	A	B	B
50 *npi287	B	B	A	B	A	B	A	B	A	A	-	-	-	A	A	B	B	A	A	B
51 *npi409	B	A	A	A	A	A	-	B	B	A	B	B	B	B	A	-	B	A	B	A
52 *npi451	B	-	-	B	B	B	B	B	B	A	A	B	B	A	A	-	A	A	-	-
53 *phi22	-	-	A	A	A	B	A	B	B	A	A	B	A	A	A	B	-	-	-	-
54 *phi93	B	A	A	B	B	A	B	A	B	A	A	B	B	B	A	A	A	A	B	A
55 *phi115	B	-	-	B	-	-	-	B	B	-	-	A	A	A	B	B	B	B	A	-
56 *php20581	B	A	-	A	A	-	A	B	-	-	-	B	B	B	A	-	-	-	-	-
57 *php4239	A	-	A	B	A	B	A	B	B	A	B	B	B	B	B	A	A	B	A	A
58 *umc102	B	B	A	B	A	B	A	A	B	-	B	-	A	A	A	-	A	B	B	A
59 *umc104a	B	B	A	A	B	A	B	A	B	A	A	A	B	A	A	B	B	B	B	B
60 *umc104b	A	B	A	A	A	B	B	B	A	A	B	A	A	A	B	B	B	B	A	B
61 *umc105	A	B	B	B	A	A	A	B	A	B	-	-	B	A	B	-	B	A	B	B
62 *umc107	A	B	A	A	A	B	B	B	A	A	B	A	A	A	B	B	B	B	A	B
63 *umc109	-	B	A	A	B	B	A	B	B	A	A	B	B	B	A	-	-	A	B	A
64 *umc110	A	A	B	A	B	B	A	-	A	B	B	A	B	A	-	B	B	A	A	A
65 *umc113	-	B	-	A	B	-	-	B	-	B	A	B	B	-	A	A	A	A	B	B
66 *umc114	A	A	A	A	A	B	A	A	A	A	B	A	A	A	-	A	A	B	A	A
67 *umc123	A	A	A	B	A	A	A	B	B	B	A	B	B	B	-	B	B	A	A	A
68 *umc124	A	B	A	A	B	A	A	B	A	B	B	A	B	A	A	B	A	B	B	B
69 *umc130	B	A	B	A	A	A	A	B	A	A	A	B	B	A	A	B	B	A	A	A
70 *umc132	A	B	A	A	A	A	A	B	A	A	A	A	A	A	B	B	A	A	A	A
71 *umc133	B	A	A	B	B	A	B	-	B	A	B	A	B	A	A	-	B	B	B	A
72 *umc135	B	A	A	B	A	B	A	B	A	A	B	A	B	A	A	A	B	A	A	A
73 *umc147	B	B	A	A	B	A	A	B	B	A	A	B	B	B	B	B	B	A	B	B
74 *umc148	B	-	A	A	B	B	A	B	B	A	A	B	B	B	A	-	A	A	B	B
75 *umc149	A	A	B	A	B	B	A	B	A	B	B	A	B	A	B	-	B	B	A	A
76 *umc14	A	A	A	A	B	A	A	B	B	-	B	-	B	-	B	-	-	B	B	B
77 *umc152	-	-	-	B	B	A	B	B	A	B	A	B	B	A	B	B	A	A	A	A
78 *umc156	A	B	A	A	-	A	B	B	A	A	-	A	B	A	A	B	B	B	B	B
79 *umc157a	A	A	A	B	B	B	A	A	B	B	A	A	A	B	B	B	B	A	B	B
80 *umc157b	-	-	A	B	B	B	A	A	B	B	A	A	A	B	B	B	B	A	B	B

Appendix E. Cont.

DNA Marker	RILs																			
	61 I68	62 I69	63 I70	64 I71	65 I72	66 I73	67 I74	68 I75	69 I76	70 I77	71 I78	72 I79	73 I80	74 I81	75 I83	76 I84	77 I85	78 I86	79 I87	80 I89
81 *umc15	B	A	A	B	B	A	B	B	B	A	A	B	B	B	A	A	A	A	B	A
82 *umc164	A	A	A	A	B	A	A	A	B	A	A	B	A	A	B	A	B	A	A	B
83 *umc166	A	B	A	B	A	B	B	B	B	A	A	B	B	B	B	A	A	A	B	A
84 *umc167	A	-	A	B	A	-	-	-	B	A	A	B	B	-	A	A	A	A	B	B
85 *umc173	B	B	A	A	B	B	A	B	B	A	A	B	B	B	A	-	A	B	B	A
86 *umc17	B	B	B	B	A	B	A	B	A	B	A	A	B	A	A	B	B	A	B	B
87 *umc186	A	A	-	B	A	A	B	B	A	A	B	A	B	A	A	B	B	B	A	A
88 *umc18	A	B	-	B	B	-	B	B	B	A	B	A	B	A	A	-	-	-	-	B
89 *umc193	A	A	-	-	B	-	B	B	B	A	A	A	B	B	A	B	B	B	B	B
90 *umc199	-	B	A	A	A	A	A	-	A	-	A	A	B	A	B	B	A	B	B	A
91 *umc19	B	B	A	A	B	A	B	A	B	A	A	A	B	B	A	B	B	B	B	B
92 *umc26	A	A	A	A	A	B	B	B	B	A	B	A	B	B	B	A	A	B	B	B
93 *umc27	B	A	A	A	B	A	B	A	B	A	A	A	B	B	A	-	A	A	B	B
94 *umc28	A	-	A	A	B	B	A	B	A	A	A	B	B	B	A	B	A	A	A	A
95 *umc29	B	-	A	B	A	A	A	A	A	A	A	B	B	A	A	B	B	A	A	A
96 *umc30	A	A	B	B	A	-	A	B	A	B	B	B	A	B	A	-	A	B	B	A
97 *umc31a	A	A	A	B	A	A	A	B	B	A	A	B	B	-	A	-	B	A	A	A
98 *umc31b	A	-	-	B	A	A	A	-	B	A	A	B	B	B	A	A	B	A	A	-
99 *umc32	A	A	A	A	B	A	B	A	A	B	A	B	B	A	A	B	B	A	A	A
100 *umc36	A	-	B	B	A	B	B	B	A	B	B	B	-	B	B	B	A	B	B	B
101 *umc37	B	B	-	-	B	B	B	B	A	B	-	-	-	B	B	B	A	B	B	A
102 *umc38	-	B	B	B	B	A	A	B	A	B	B	A	A	A	B	A	B	B	A	B
103 *umc39	-	-	-	-	-	-	-	B	B	-	B	-	-	-	B	-	-	B	-	-
104 *umc45	A	A	A	B	A	B	A	A	A	A	A	A	B	B	B	B	B	A	-	B
105 *umc48	-	-	-	B	A	B	A	B	A	B	B	B	A	B	A	A	A	B	B	-
106 *umc50	B	B	-	B	A	B	A	-	B	A	-	B	B	A	B	A	A	B	-	-
107 *umc51	A	A	A	A	B	B	A	A	B	A	A	A	B	B	A	B	B	B	-	A
108 *umc55	A	B	A	A	A	A	A	B	B	B	B	A	B	A	A	-	A	A	B	B
109 *umc59	A	A	A	A	B	B	A	B	A	A	B	B	A	A	A	B	B	B	A	A
110 *umc5	A	A	-	-	A	B	A	A	B	B	B	A	B	B	-	A	-	-	B	B
111 *umc65	A	B	A	A	A	A	A	A	B	B	A	A	A	A	B	B	B	B	B	B
112 *umc66	A	B	A	A	B	A	B	A	B	A	A	A	B	A	A	-	B	B	B	B
113 *umc67	A	B	A	A	A	B	B	B	A	A	B	A	A	A	B	B	B	B	A	B
114 *umc72	A	A	A	A	A	A	A	A	B	B	A	B	B	A	B	-	A	A	B	A
115 *umc76	-	A	A	-	B	B	B	-	-	B	B	A	B	B	-	B	A	B	A	-
116 *umc80	B	A	-	B	A	-	-	B	A	A	B	B	B	B	A	B	A	B	-	A
117 *umc89	A	A	B	B	-	A	B	B	B	B	A	A	A	-	A	B	B	A	A	A
118 *umc92	B	B	A	B	A	B	A	A	B	A	A	B	A	A	B	A	A	A	A	A
119 *umc96	B	B	-	A	A	B	B	B	A	B	A	A	B	A	-	-	-	-	-	-
120 *umc97	B	-	A	-	B	B	B	A	B	A	A	A	B	A	B	A	B	A	A	-

Appendix E. Cont.

	RILs																			
DNA Marker	81 I91	82 I92	83 I93	84 I94	85 I95	86 I96	87 I97	88 I98	89 I99	90 I100	91 I101	92 I102	93 I103	94 I104	95 I105	96 I106	97 I107	98 I108	99 I109	100 I110
1 *asg30	B	A	A	A	A	B	A	A	A	B	A	A	B	B	A	A	A	-	A	B
2 *asg62	A	A	B	A	B	A	A	B	B	B	A	B	B	B	B	B	B	A	B	B
3 *asg75	B	A	A	A	A	B	A	A	B	-	A	A	B	B	A	A	A	B	A	B
4 *bnl10.24	B	A	A	B	B	A	A	B	B	B	B	A	B	B	B	A	A	B	A	B
5 *bnl12.09	A	A	B	B	B	A	A	B	A	A	A	A	B	A	B	A	B	B	A	A
6 *bnl12.30	A	A	A	A	A	B	B	A	A	B	A	A	A	A	-	B	B	B	A	A
7 *bnl12.30b	B	A	B	B	B	B	A	A	A	B	B	B	A	A	A	A	B	B	B	A
8 *bnl13.05	B	B	A	A	B	A	B	B	A	A	A	B	A	A	B	B	B	B	A	B
9 *bnl16.06	B	A	A	A	B	A	B	B	B	B	B	A	A	B	A	A	A	B	A	B
10 *bnl3.04	B	B	B	A	B	B	A	A	A	A	A	B	A	B	A	A	B	B	B	A
11 *bnl4.06	B	A	B	A	B	A	A	B	A	B	A	A	A	A	B	B	B	B	B	B
12 *bnl5.40	B	B	A	B	B	B	A	A	B	B	B	A	A	A	A	B	A	B	A	B
13 *bnl5.46	B	B	A	-	A	B	A	A	A	B	A	-	A	B	A	B	B	B	B	-
14 *bnl5.62	-	-	B	A	-	-	B	B	B	A	A	A	A	A	B	A	A	A	B	A
15 *bnl6.25	A	A	-	-	-	A	A	-	-	A	A	A	A	B	-	-	B	-	B	A
16 *bnl7.49	B	B	B	A	B	A	-	A	A	-	A	A	A	A	A	-	B	B	-	-
17 *bnl7.71	B	A	B	A	B	A	A	B	A	B	A	A	A	A	B	B	B	B	B	B
18 *bnl8.01	B	A	A	B	B	A	A	B	B	B	B	A	-	B	B	A	A	B	A	B
19 *bnl8.17	A	A	B	B	B	B	B	B	A	B	A	A	A	A	B	A	B	A	B	A
20 *bnl8.39	A	B	A	A	A	A	A	A	B	A	B	B	A	B	B	A	A	B	B	B
21 *bnl8.45	B	B	B	A	A	B	A	B	A	A	A	B	A	B	A	B	B	B	B	B
22 *csu110	B	A	A	B	A	A	A	A	A	A	B	A	B	A	B	A	A	B	A	B
23 *csu11	A	B	B	A	A	A	B	B	B	B	A	B	A	B	A	B	A	B	B	B
24 *csu12	B	A	B	A	A	A	B	-	A	A	B	B	A	B	B	B	-	-	-	-
25 *csu136	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A
26 *csu13	B	A	A	B	A	A	B	B	B	B	A	A	A	A	B	B	B	B	B	A
27 *csu145	-	-	-	B	B	-	A	A	B	B	-	-	B	A	A	A	A	B	A	A
28 *csu146	B	B	A	B	A	B	B	A	B	A	A	B	B	A	B	A	B	B	B	B
29 *csu164	B	A	B	A	A	B	A	B	B	A	A	A	A	A	A	B	A	A	A	-
30 *csu25	B	B	B	A	B	B	A	A	A	A	A	B	A	B	A	A	B	B	B	A
31 *csu29	B	A	A	A	B	-	B	A	B	-	A	-	A	A	B	-	A	A	-	-
32 *csu31	A	B	A	A	A	A	B	-	A	A	A	A	A	B	A	A	A	A	A	B
33 *csu36	A	A	A	A	A	B	A	A	A	B	A	A	A	-	A	B	B	-	A	B
34 *csu39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35 *csu46	B	A	A	A	-	B	A	B	B	A	B	B	A	B	A	B	B	B	-	A
36 *csu50	B	A	B	B	A	A	B	B	A	A	-	B	A	A	B	B	A	B	A	B
37 *csu54	B	B	B	B	-	B	B	A	B	A	A	A	B	B	B	B	A	B	A	B
38 *csu59	A	A	B	B	B	A	B	B	B	B	B	A	B	B	B	B	-	B	B	A
39 *csu61	-	-	-	-	-	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40 *csu92	B	A	A	A	B	B	A	A	B	A	B	B	-	-	B	B	A	B	A	-

Appendix E. Cont.

DNA Marker	RILs 81 I91	82 I92	83 I93	84 I94	85 I95	86 I96	87 I97	88 I98	89 I99	90 I100	91 I101	92 I102	93 I103	94 I104	95 I105	96 I106	97 I107	98 I108	99 I109	100 I110
41 *csu95a	B	B	B	B	A	B	B	A	B	B	B	A	A	A	A	B	B	B	A	A
42 *csu95b	B	A	A	A	A	A	A	B	B	A	A	A	B	A	A	A	A	A	B	B
43 *mag1f03	B	B	A	B	A	B	A	A	B	B	A	B	A	B	B	B	B	B	B	B
44 *npi105	B	B	B	B	B	A	A	B	B	A	A	B	A	B	A	B	B	B	B	A
45 *npi220	A	B	A	A	A	A	A	A	-	-	-	B	A	B	B	A	A	-	B	B
46 *npi232	B	B	A	A	A	B	B	B	B	A	B	A	A	B	A	B	B	B	B	B
47 *npi238	B	A	A	B	B	B	A	B	A	B	A	A	A	A	-	A	A	B	A	B
48 *npi239	B	B	B	B	B	A	A	B	A	A	A	A	B	B	A	B	-	-	A	A
49 *npi285	B	A	A	A	A	B	A	A	A	A	B	B	A	B	A	B	B	B	B	A
50 *npi287	B	A	B	A	B	A	B	A	B	A	B	A	A	B	A	B	B	B	B	B
51 *npi409	A	A	B	B	B	A	A	B	B	A	A	A	A	B	A	B	B	B	B	A
52 *npi451	A	B	B	A	A	A	A	B	A	B	A	B	A	A	A	B	B	B	B	B
53 *phi22	-	-	-	-	-	-	-	-	B	B	B	A	B	A	A	A	B	B	B	A
54 *phi93	A	B	B	A	A	A	B	B	A	A	B	B	A	B	A	B	-	-	-	-
55 *phi115	A	B	A	A	A	A	B	A	A	A	A	A	B	B	B	A	-	-	A	-
56 *php2058	B	A	A	-	-	-	B	-	-	B	-	A	B	-	-	A	B	B	A	A
57 *php4239	B	A	A	B	A	A	B	B	A	A	-	B	A	A	B	A	A	B	A	-
58 *umc102	A	B	A	A	A	A	A	A	A	A	A	B	A	B	B	A	A	A	B	B
59 *umc104	B	B	B	A	A	B	B	B	B	A	A	B	A	B	B	B	B	B	A	B
60 *umc104	B	A	B	B	B	B	A	B	B	A	A	A	A	A	A	B	A	-	A	B
61 *umc105	B	B	-	A	B	A	B	B	A	-	B	A	-	B	-	-	-	-	B	-
62 *umc107	B	A	B	B	B	B	A	B	B	A	A	A	A	A	A	B	A	A	A	B
63 *umc109	B	B	B	B	A	B	B	A	B	B	B	A	A	A	A	B	-	-	A	A
64 *umc110	A	A	-	-	-	A	B	B	B	B	A	A	B	A	B	A	A	B	B	A
65 *umc113	-	-	B	A	A	-	-	A	-	-	-	A	A	A	B	-	-	B	A	A
66 *umc114	A	A	A	B	A	A	A	A	B	A	B	B	B	B	B	B	A	A	A	B
67 *umc123	A	A	A	A	B	A	A	B	A	A	B	A	A	A	-	B	A	B	A	-
68 *umc124	A	A	B	B	B	A	A	A	A	A	A	A	B	A	B	A	B	B	A	A
69 *umc130	B	-	B	A	A	-	A	B	B	A	B	B	A	B	A	B	B	B	B	A
70 *umc132	A	A	B	A	B	A	A	A	A	A	B	A	B	A	A	A	A	A	A	A
71 *umc133	-	B	B	A	A	A	A	B	A	A	A	B	A	B	A	B	B	B	B	B
72 *umc135	B	A	A	B	B	A	A	B	B	B	A	B	A	B	A	A	A	A	B	B
73 *umc147	A	A	B	A	B	A	A	B	B	B	B	A	B	B	A	B	B	B	B	A
74 *umc148	B	B	B	B	A	B	B	A	B	B	B	A	A	A	A	B	B	B	A	A
75 *umc149	A	A	A	A	A	A	B	B	B	B	A	A	A	A	B	A	A	B	B	B
76 *umc14	B	-	B	B	B	B	-	A	-	A	-	A	B	A	B	B	B	B	A	B
77 *umc152	A	B	-	A	-	-	B	-	A	A	A	A	B	B	B	A	B	B	A	B
78 *umc156	B	A	A	A	B	B	B	A	B	B	A	B	B	A	B	A	A	B	B	A
79 *umc157	B	A	B	A	B	A	B	B	B	B	A	A	B	A	A	A	A	A	A	A
80 *umc157	B	A	B	A	B	A	B	-	B	B	A	A	B	A	A	A	A	-	-	-

Appendix E. Cont.

	RILs																			
DNA	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
Marker	I91	I92	I93	I94	I95	I96	I97	I98	I99	I100	I101	I102	I103	I104	I105	I106	I107	I108	I109	I110
81 *umc15	-	-	B	A	A	A	B	B	A	A	-	B	A	B	A	B	B	B	B	B
82 *umc164	A	A	B	A	B	A	B	B	B	A	A	A	B	A	B	B	A	A	B	A
83 *umc166	A	A	B	A	A	A	A	B	A	B	A	A	B	B	B	B	A	B	A	B
84 *umc167	A	A	-	A	A	A	A	-	A	-	-	-	-	-	-	-	A	B	B	B
85 *umc173	B	B	B	B	A	B	B	A	B	B	B	A	A	A	A	B	B	B	A	-
86 *umc17	B	B	A	A	B	A	B	B	A	B	B	A	B	B	A	A	A	B	B	B
87 *umc186	B	A	A	B	A	A	B	A	B	B	A	B	A	A	B	B	B	B	B	B
88 *umc18	-	A	A	B	A	A	A	A	A	A	B	A	B	A	B	A	A	B	A	B
89 *umc193	B	A	A	A	A	B	B	A	B	A	B	B	B	A	B	A	A	B	B	A
90 *umc199	A	A	A	B	-	B	B	A	A	A	A	A	-	A	-	-	-	B	-	-
91 *umc19	B	B	B	A	A	B	B	B	B	A	A	B	A	B	B	B	B	B	A	B
92 *umc26	A	B	B	A	B	B	A	A	A	B	B	B	A	A	A	B	A	B	B	B
93 *umc27	B	A	B	A	B	A	A	B	A	B	B	A	A	B	B	B	B	B	B	B
94 *umc28	A	B	A	A	A	B	B	A	B	B	B	B	A	A	A	B	A	A	A	A
95 *umc29	B	A	A	A	B	A	B	B	B	A	B	A	A	B	A	B	B	B	B	B
96 *umc30	A	-	A	A	A	A	B	B	B	B	A	A	A	B	A	-	-	-	A	B
97 *umc31a	B	A	A	A	B	B	A	A	B	A	B	B	B	B	B	B	A	B	A	A
98 *umc31b	B	A	-	A	-	B	A	A	B	A	B	B	-	B	B	B	A	B	A	A
99 *umc32	B	A	A	A	A	A	B	A	B	A	B	A	A	A	A	A	A	B	B	A
100 *umc36	B	B	A	B	A	A	B	B	B	A	A	B	B	B	A	A	A	-	-	-
101 *umc37	A	B	A	A	A	A	B	B	B	A	A	A	B	B	-	A	B	B	A	B
102 *umc38	B	A	A	B	B	B	B	A	B	A	A	A	A	A	A	A	B	A	B	B
103 *umc39	A	B	B	-	-	-	-	-	-	-	-	-	B	A	A	B	B	-	A	B
104 *umc45	B	A	A	A	B	A	B	A	B	B	B	A	A	B	A	B	B	B	-	-
105 *umc48	A	B	A	A	A	A	B	-	B	B	A	A	-	B	A	A	A	-	A	B
106 *umc50	-	-	-	-	-	-	-	-	B	A	B	-	-	B	B	A	A	-	B	B
107 *umc51	B	B	A	B	B	A	-	A	B	-	B	A	B	A	B	A	B	A	B	B
108 *umc55	B	B	B	B	A	B	A	A	B	A	A	A	A	B	B	B	A	A	B	A
109 *umc59	B	B	A	A	A	B	A	A	B	A	A	B	A	A	A	A	A	A	B	B
110 *umc5	-	-	-	-	-	-	-	A	B	B	A	A	A	A	B	B	A	B	A	B
111 *umc65	B	A	A	A	A	B	A	A	B	A	A	A	A	A	A	A	A	A	B	B
112 *umc66	B	B	B	-	A	B	B	B	B	A	-	B	A	B	B	B	A	B	A	B
113 *umc67	B	A	B	B	B	B	A	B	B	A	A	A	A	A	A	B	A	A	A	B
114 *umc72	A	A	B	A	B	A	A	B	B	B	B	A	A	A	A	B	B	B	A	B
115 *umc76	A	A	-	B	-	-	-	-	-	A	-	-	B	A	B	-	A	B	A	A
116 *umc80	B	A	A	B	A	B	A	A	B	B	A	B	A	-	B	A	A	B	B	A
117 *umc89	-	B	A	A	-	A	B	-	-	-	-	-	-	-	-	-	-	-	-	-
118 *umc92	A	B	A	A	A	A	A	A	B	A	B	B	B	B	B	B	A	A	A	B
119 *umc96	A	A	A	-	-	B	A	B	-	B	A	A	A	B	-	B	-	B	-	-
120 *umc97	B	-	B	B	B	B	A	-	A	B	B	B	B	A	B	B	B	-	B	A

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