RESTRICTION FRAGMENT LENGTH POLYMORPHISM ANALYSIS OF HOST-PLANT RESISTANCE TO FOUR MAIZE PATHOGENS

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

AGRONOMY AND SOIL SCIENCE

AUGUST 1995

By

Reiguang Ming

Dissertation Committee

James L. Brewbaker, Chairman
James A. Silva
Chung-Shih Tang
Chifumi Nagai
Scot C. Nelson

UMI Number: 9604170

UMI
300 North Zeeb Road
Ann Arbor, MI 48103

DEDICATION

This dissertation is dedicated to my grandparents, Pingxiang Ming and Ke Wu, who set me on the right path in my childhood with patience, trust, and encouragement. They are living examples of unconditional love and support.

ACKNOWLEDGMENTS

The list continues to grow of people to whom I would like to express my appreciation for advice, support, and technical assistance in making my dissertation possible. I am grateful to my advisor Dr. James Brewbaker for initiating the project and offering advice and encouragement through the years. I am especially grateful for providing me the opportunities to work at Ohio State University (Wooster) and the University of Missouri (Columbia), which broadened my experience and opened new doors for me. I am thankful to Dr. Chifumi Nagai for her advice in initiating the project and her help and understanding through the years. Much appreciation and thanks are also extended to my other committee members, Dr. James Silva, Dr. Chung-Shih Tang, and Dr. Scot Nelson for their consistent advice and support throughout my studies.

Special thanks go to Dr. Michael McMullen, USDA/ARS, University of Missouri, for generously providing the lab supply and also for his advice and easy-going style during the two semesters I was working with him. Without his support, I could not have completed this work. Appreciation also goes to Dr. Richard Pratt for his advice and hospitality when I was in Ohio.

I am grateful to Hawaii Sugar Planter's Association, Northrup King Seed Company, and Hawaii Natural Energy

Institute for providing the financial support for my research.

Dr. Hyeon Gui Moon's work, providing the foundation for my research, is highly acknowledged. Many thanks go to my colleagues Weiguo Sun and Guohua Zan, who made my life much easier in Hawaii. I can't forget Drs. Michael Austin and Charles Sorensson for their friendship, positive attitude, and English editing through the years. I also can't leave Jon Kamemoto out, who always created a joyful atmosphere.

Thanks are also extended to Drs. Kevin Simcox, Denis Kyetere, Patrick Byrne, Suresh Sondur for their valuable inspiration and suggestions. The technical assistance from Mark Jones, Brenda Schult, Theresa Musket, and Guiling Xu is greatly appreciated.

And last but not the least, appreciation goes to Lynne Horiuchi for her help with paper work and her warm heart. Thanks also to the staff members of the Departments of Horticulture and Agronomy and Soil Science of the University of Hawaii at Manoa and of the Waimanalo Experimental Research Station.

ABSTRACT

Restriction fragment length polymorphism (RFLP) markers were used to investigate host plant resistance to four maize pathogens in 117 maize recombinant inbred lines (RILs), derived from the cross of Hi31 (a B68 conversion) and Ki14 (a Thai inbred). The four pathogens are maize mosaic virus (MMV), *Erwinia stewartii*, *Puccinia polysora*, and *Exserohilum turcicum*. The phenotypic data of RILs were analyzed with 127 RFLP loci using MAPMAKER/QTL and single factor analysis of variance.

Ninety-one RILs were evaluated for MMV resistance in a disease nursery in Hawaii in the summer of 1994. Fifty RILs were classified as susceptible and forty one as resistant. Loci on chromosome 3 near the centromere showed the largest effects, indicating that a major MMV resistance gene was located in this region. This gene, previously named *mv*, was mapped on chromosome 3, 4 cM and 5.6 cM from RFLP markers *umc*102 and *php*20508, respectively.

Seventy one RILs and ten sub-lines of each parent were planted at Henderson, Kentucky for evaluation and were naturally infected by *E. stewartii*. Thirty eight RILs were classified as resistant and thirty three as susceptible. A major gene conferring resistance to *E. stewartii*, designated *sw1*, was mapped on the short arm of chromosome 1, 4 cM and 8.2 cM from RFLP markers *umc167* and *umc67*, respectively.

One hundred and seventeen RILs and twenty sub-lines of each parent were planted at Waimanalo, Hawaii and one hundred and seven RILs were planted at Mindanao, The Philippines to evaluate *P. polysora* resistance under natural infection. Five QTLs were mapped on chromosomes 2, 4, 6, 9, and 10. The QTL on chromosome 6 appeared to play a particularly important role in conditioning race-nonspecific resistance to *P. polysora*.

One hundred and ten RILs and ten sub-lines of each parent were evaluated for race-nonspecific resistance to *E. turcicum* at Mealani, Hawaii in 1993. A clear 1:1 segregation for general resistance characterized RILs in this nursery. Ninety five RILs were planted in two separate trials to evaluate the response to race 0 and race 1 of *E. turcicum*, respectively, at Urbana, Illinois in 1994. The QTL located on chromosomal region 3S was important in conferring the race-nonspecific resistance to *E. turcicum*.

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GENERAL INTRODUCTION

Plant diseases have an enormous negative impact on agricultural crop production throughout the world. Generations of agronomists and plant pathologists have devoted considerable effort toward controlling plant diseases. Traditional plant breeding methodology has been successfully applied to develop resistant varieties and hybrids for agronomically important crops. In addition, standard techniques of plant pathology have been important tools to control plant diseases, including quarantine, eradication, crop rotation, and certified pathogen-free stock. The disadvantages of these traditional methods are the expense, questionable effectiveness, and lack of reliability. In the past decade, the tools of molecular biology have permitted rapid mapping and transformation of plant disease resistance genes. Many plant diseases may be controlled either by accelerated conversion of resistance genes into plants through DNA-based techniques, or by genetically engineering microorganisms that can effectively antagonize or compete with particular pathogens.

Restriction fragment length polymorphism (RFLP) is one of the most important and immediate applications of modern molecular methods to plant genetics and breeding. RFLP

markers are cloned DNA segments that can be used to reveal base pair changes or rearrangements in homologous DNA sequences (Beckman and Soller 1983; Tanksley *et al.*, 1989). The use of RFLP markers has led to the rapid construction of linkage maps in many plant species and the placement of genes that control qualitative and quantitative traits onto these maps (Tanksley, 1993).

The objectives of this study were: 1) to map and characterize the maize mosaic virus resistance gene, *mv,* as well as other quantitative trait loci (QTLs) associated with MMV resistance in maize; 2) to map genes or QTLs conferring resistance to other maize pathogens, including *Erwinia stewartii, Exserohilum turcicum,* and *Puccinia polysora,* which were segregating in this RIL population (Hi31 X Ki14); and 3) to identify RFLP markers flanking the disease resistance gene(s) or QTLs that could be used in a breeding program for RFLP marker-assisted selection. Due to the economic importance of the disease resistance genes, mapping these genes would be very useful for both plant breeding and molecular genetic study.

CHAPTER 1

LITERATURE REVIEW

1.1. Recombinant Inbred Lines in Quantitative Traits Studies

Recombinant inbred lines (RILs) are produced by selfing or sibbing the progeny of an F_2 derived from two unrelated inbreds. After five to six generations of inbreeding to achieve homozygosity, the RILs become fixed for short linkage blocks of progenitor alleles. If a trait is controlled by a single gene, then a 1:1 segregating ratio can be expected in the RIL population. Recombinant inbred lines (as single seed descent lines) were used in soybean breeding (Brim, 1966). Bailey (1971) used RILs to analyze histocompatibility (*H*) and other gene systems in mice. Jenkins *et al.* (1981) used RILs in mice for linkage studies and concluded that the *dilute* mutation for mouse coat was the result of the integration of an ectopic virus. RILs have also been used for estimations of the components of variance in plants (Jinks, 1981), and for the mapping of legumin genes in *Pisum* (Domoney *et al.*, 1986).

More recently, a genetic map based on restriction fragment length polymorphism (RFLP) has been produced in maize using two RIL populations (Burr *et al.*, 1988). The two RIL populations were derived from F_2 populations of T232 X CM37 and CO159 X Tx303. This map covered the entire maize genome including 240 loci, and has been expanded to more than 600 loci (Matz *et al.*, 1995). Wang *et al.* (1994) developed a series of RILs in rice and mapped two major genes for complete resistance and ten QTLs for partial resistance to blast disease with RFLP markers. Kyetere (1995) typed a RIL population in maize developed at the University of Hawaii by Moon (1995) derived from two tropical lines, Hi34 and Tzi4, with 87 RFLP markers. Kyetere was able to map a major gene conferring resistance to maize streak virus on the short arm of chromosome 1.

Moon (1995) developed nine sets of RILs from ten parents of tropical and temperate origin to study quantitative trait loci (QTLs) controlling disease resistance as well as morphological traits. These RILs were self-pollinated using the single seed descent method until the S_6 generation. A total of 1068 RILs, 100 to 142 RILs in each set, were produced. Six RIL populations were evaluated together with their parental sub-lines at seven locations in three countries. Evaluations included resistance to eight

diseases and two pests. The eight diseases were southern rust, common rust, northern leaf blight, southern leaf blight, bacterial leaf blight, Stewart's bacterial wilt, maize mosaic virus, and maize streak virus. The two pests were parasitic flowering plant (Striga) and European corn borer (*O. nubilalis*). The normal distribution curve approach (Brewbaker, 1993) was used to analyze the QTLs conferring resistance to these diseases and pests. Moon concluded that the general resistances to these eight diseases fitted the expected 1:1 monogenic segregation and were therefore controlled by one major QTL. The resistances to the two pests, however, were suggested to be governed by two major unlinked QTLs. Five RIL populations were used to study the QTLs influencing ten morphological and agronomic traits at three locations in 1993 and 1994. Moon concluded that five traits, including ear height, days to silking, stalk internode length, stay green, and root lodging, were controlled by a major QTL in the RIL populations understudy. Another four traits appeared to be governed by two unlinked QTLs; these were plant height, number of leaves, number of tassel branches, and central spike length of tassel.

1.2. Restriction Fragment Length Polymorphism (RFLP) in Plant Genetic Improvement

1.2.1. Theory of RFLP

The molecular basis of polymorphisms observed in the length of the restriction fragments is most often due to single-base substitutions in DNA that create or abolish recognition sites for a restriction enzyme. The enzymes that are commonly used for RFLP analysis require four - six base pair (bp) recognition sequences. Cleavage frequency can be estimated by making the assumption that each of the different nucleotides occurs randomly and in equal amounts for a given DNA sequence. A 6-bp recognition enzyme would be expected to cut DNA once every 4^6 or 4096 bp. A 4-bp recognition enzyme would be expected to cut DNA once every 256 bp. If the maize genome size is considered 5×10^9 bp (Walbot and Messing, 1988), the potential polymorphisms which can be used as markers are unlimited.

The presence of specific fragments (RFLP) in an individual can be tested by cutting the DNA with a restriction enzyme, separating the fragments based on size using agarose gel electrophoresis, transferring the DNA to a cellulose membrane, and hybridizing DNA fragment with the ^{32}P

labeled DNA probe homologous to the fragment. DNA probes that include a highly repetitive DNA sequence are not suitable as they hybridize with a large number of DNA fragments resulting in a continuous smear. Therefore, unique DNA sequences are generally used as probes in determining RFLPs.

In most eukaryotes, a large proportion of genomic DNA sequences is repeated many times. These repeats are often interspersed with unique sequence DNA. The presence of repetitive sequences makes it necessary to select unique DNA sequences for use as probes in RFLP analysis. Currently the two methods described below are used to obtain unique sequences.

1.2.1.1. Method A. cDNA Clones

Classical and molecular genetic studies indicated that a majority of the genes that are transcribed into mRNA are in single or low copy numbers (Tanksley and Pichersky, 1988a). Complementary DNA (cDNA) clones derived from gene transcripts are therefore a good source of low-copy DNA. Probes corresponding to mRNAs are made by reverse transcription followed by cloning of the resultant DNA. Bernantzsky and Tanksley (1986) have reported that, in

tomato, out of 34 cDNA clones picked at random, 53% corresponded to single copy genes, 32% corresponded to two genetically independent loci, and 3-5% of the clones attached to multiple sites in the genome.

The length of the cDNA clones is limited (less than 1 kilobase), and consequently the development of autoradiographs may take longer exposure period because of weak signal. Helentjaris *et al.* (1986) have reported that more than half of the cDNA probes used in maize resulted in very weak hybridization signals and were unfit for DNA analysis. The cDNA probes map only transcribed regions, hence, they may be non randomly distributed over the chromosome. It is possible that this could accentuate the distortion between the genetic map and physical map.

1.2.1.2. Method B. Genomic Clones

The problems mentioned above are overcome by developing probes from genomic DNA. For most plant species, however, the majority of random genomic clones are likely to carry repeated sequences making them useless. Tanksley *et al.*, (1988b) suggested a two-step process to obtain genomic clones of unique sequences: 1) Much of the DNA in eukaryotes is highly repeated and highly methylated at cytosines.

High-copy DNA contains more methyl cytosine than low-copy DNA. The proportion of low copy DNA in a digest could be enhanced by using a restriction endonuclease that acts only on unmethylated portions of the genome (such as *Pst*I), recognizing and cutting only sequences in which cytosine is not methylated. Tanksley *et al.* (1988b) found that 92% of the *Pst*I digested clones corresponded to sequences present only once in the genome, whereas the majority of the *Eco*RI digested clones of comparable size had only 35% of low-copy DNA; 2) Large numbers of bacterial colonies can be grown directly on nylon hybridization filters, each of the colonies harboring a plasmid into which plant DNA has been cloned. The colonies are lysed on the filter, the denatured plasmid DNA is bound to the filter, and the filter is then probed with nick-translated, total nuclear DNA for 12-24 hours. The concentration of a repetitive sequence in the genomic probe is relatively great and would result in a strong signal when it was used to probe a clone consisting of a complementary, highly reiterated sequence. Conversely, a low-copy number clone would give a very weak signal when it was probed with genomic DNA. Figdore *et al.* (1988) reported a high proportion (75-87%) of unique sequence by following this stepwise procedure for obtaining low-copy genomic DNA fragments.

1.2.2. RFLP in Plant Genetic Improvement

The single gene markers used in higher plant genetics were morphological markers before the discovery of molecular markers. The first genetic marker used in QTL genetic study was seed-coat color which associated with the seed size in beans (*Phaseolus vulgaris*) (Sax, 1923). Sax interpreted the association as the linkage of the single gene controlling seed color with one or more of the polygenes controlling seed size. Wexelsen (1933) reported the association of the length of internode of the spike and texture of the awn (rough or smooth) in barley. Rasmusson (1935) demonstrated the linkage of the flowering time and flower color. Everson and Schaller (1955) found morphological markers which flanked a chromosomal region affecting yield in barley. Brewbaker (1974) suggested the linkage of maize mosaic virus resistance gene and two morphological markers, lg_2 and na_1, in maize. Thoday (1961) suggested that since single gene markers were distributed throughout the genome of an organism, it should be possible to map and characterize all of the QTLs affecting a trait. Two factors limited putting Thoday's idea in practice: 1) these phenotypic markers are limited in number; and 2) morphological markers tend to be

associated with undesirable phenotypic effects (Tanksley, 1989).

The first molecular markers used in genetic studies were isozymes (Hunter and Markert, 1957), which are protein-based. Although isozyme loci have proven to be quite useful, more extensive application is limited by an insufficient number of marker loci and their general lack of informativeness (Burr *et al.*, 1988).

A more useful molecular marker is DNA-based restriction fragment length polymorphism (Grodzicker *et al.*, 1974). RFLP is based on cloned sequences of DNA that can be genetically mapped using traditional linkage analysis. The advantages of RFLP compared with morphological markers include: 1) the number of RFLPs is potentially unlimited for any plant species; 2) RFLP markers behave in a codominant manner that allows the genotypic identification of all individuals in a segregating population; 3) RFLP markers are phenotype-neutral and do not interfere with any phenotypic expression; 4) the level of allelic variation for RFLP markers is much higher than morphological markers; and 5) RFLP markers are free of epistatic effects and genotype-environment interaction.

Grodzicker *et al.* (1974) first used RFLP to map temperature-sensitive mutants of adenovirus in humans.

Botstein *et al.* (1980) explored the application of constructing an RFLP genetic map in humans. Since then, many applications of RFLP have been used in plant improvement. In the past decade, RFLP maps have been constructed for many plant genera such as maize (Helentjaris *et al.*, 1986; Burr *et al.*, 1988; Hoisington and Coe, 1989), rice (McCouch *et al.*, 1988), barley (Heun, 1991), wheat (Sharp *et al.*, 1989), soybean (Muehlbauer *et al.*, 1991), sorghum (Whitkus, 1992), tomato (Tanksley *et al.*, 1988; Paterson *et al.*, 1988), lettuce (Landry *et al.*, 1985), potato (Gebhardt *et al.*, 1989), and Arabidopsis (Chang *et al.*, 1988). RFLPs were widely used in mapping genes that control qualitative and quantitative traits on these maps.

RFLP linkage maps are most useful to locate economically important genes. Many disease resistance genes have been mapped with tightly linked RFLP markers. These include downy mildew (*Bremia lactuca*) in lettuce (Landry *et al.*, 1987), *Fusarium oxysporum* in tomato (Sarfatti *et al.*, 1989), powdery mildew (*Erisyphe graminis*) in barley (*Hinze et al.*, 1991), *Phytophthora* rot in soybean (Diers *et al.*, 1991), leaf blast (*Magnaporthe grisea*) in rice (Yu *et al.*, 1991), northern corn leaf blight (*Exserohilum turcicum*) race 1 (Bentolila *et al.*, 1991) and race N (Simcox and Bennetzen, 1993) in maize, common rust *Puccinia sorghi 3* (*rp3*) in maize

(Richter, personal communication), maize dwarf mosaic virus (McMullen and Louis, 1989), wheat streak mosaic virus in maize (McMullen *et al.*, 1991; 1994), and maize streak virus (Kyetere, 1995).

Most of the agriculturally important traits are quantitatively inherited, such as yield, quality, and general disease resistance. These traits are often controlled by genes or QTLs at several unlinked loci and are therefore more difficult to manipulate in breeding. Classical breeding based on phenotypic selection could be ineffective due to multiple segregation loci and environmental variation. Using RFLPs, the QTLs underlying several agronomic traits have been identified. When QTLs of interest have been mapped relative to tightly linked RFLP markers, selection can be based on screening for RFLP markers rather than for the plant phenotype. Such marker-assisted selection may improve genetic gains in breeding programs. In maize, QTLs for yield and yield components have been investigated (Edwards *et al.*, 1992, Stuber *et al.*, 1992, Zehr *et al,*. 1992, Ajmone-Marsan *et al.*, 1994). QTLs influencing kernel oil concentration have been identified (Goldman *et al.*, 1994). The most comprehensive study on mapping QTLs for heterosis was reported by Stuber *et al.* (1992). RFLP has also been used to explore QTLs important

for understanding the evolution of maize from teosinte (Doebley and Stec, 1993). QTLs for plant height were shown to be located in proximity to major genes that condition plant height (Beavis *et al.*, 1991, Ajmone-Marsan *et al.*, 1994). The data support Robertson's hypothesis that loci with qualitative mutants are often the same loci that affect the expression of quantitative traits, and that the qualitative mutants are mostly null or near-null alleles at QTLs (Robertson, 1985).

1.3. Mapping Populations

1.3.1. Recombinant Inbred Lines

Recombinant inbred lines (RILs) are useful for mapping QTLs since they represent a stable source of germplasm and comprise a random set of combinations of genes from their two parents. Mapping in this population is based on the assumption that when two loci are tightly linked, their parental alleles will occur together in direct proportion to the linkage distance.

There are several major advantages of RILs over F_2 and backcross populations in gene and QTL mapping (Bailey, 1981, Burr *et al.*, 1988). The first is that RILs can be

propagated indefinitely without further segregation, once homozygosity has been reached. The same population for mapping can be used by different investigators and evaluated in a range of environments. In contrast, F_2 and backcross populations of annual plants can be evaluated only once and the DNA extracted from them will be eventually exhausted. To resume mapping, the whole set of marker loci will have to be genotyped again in a new segregating population.

The second advantage is that RILs permit a new marker to be mapped easily by comparing data with those of previously mapped markers. All information obtained from mapping in RIL populations is cumulative and RILs permit follow-up and confirmation studies.

A third advantage is that RILs can be evaluated in many different environments. Since each genotype is represented by an inbred line rather than by an individual plant, a more accurate assessment can be made of the genetic component of variance in quantitative traits. One disadvantage of developing new recombinant inbred lines is the time required for their construction. This is minimized in an environment like that of Hawaii and may be overcome by using previously existing breeding materials.

1.3.2. F_2 and Backcross Populations

Studies of F_2 and backcross populations start with the identification of two parental genotypes, preferably inbred lines homozygous for alternate alleles at a number of loci. The F_1 hybrids are then generated by crossing these two inbreds. The classic schemes continue with either selfing or backcrossing to generate a segregating F_2 or backcross population. These populations can be evaluated for phenotypic performance and genotyped with RFLP markers. The population size is a direct function of the requirement for small and more accurate estimation of map distances. To achieve fine mapping, the population size needs to be fairly large (from several hundreds to thousands of individual offspring). Basically, the fine mapping can only be done with F_2 or backcross populations due to the population size required.

A maize RFLP genetic map was constructed and a set of core RFLP markers was selected for mapping genes and QTLs using an immortalized F_2 population (Hoisington and Coe, 1989; Gardiner *et al.*, 1993). Immortalized F_2 signifies a set of mapping atrains derived by a procedure that maintains (i.e., "immortalizes") the heterozygous alleles in each individual F_2 plant by pooled random mating in the F_3 and

subsequent generations. McMullen and Louie (1989) mapped maize dwarf mosaic virus strain A resistance gene *mdm1* to the short arm of chromosome 6, which is tightly linked to and located between the RFLP marker loci *umc85* and *bnl6.29*. McMullen *et al*. (1991, 1994) mapped wheat streak mosaic virus resistance gene *wsm1* to the short arm of chromosome 6 tightly linked to *umc85*, *wsm2* and *wsm3* on chromosomes 3 and 8 respectively. The mapping of *wsm1* near *mdm1* shows that these genes are either allelic or are tightly linked. Simcox *et al*. (1995) obtained a fine map for *mdm1* with 1488 backcross plants and 7650 F_2 plants.

1.3.3. Near-Isogenic Lines

Near-isogenic lines (NILs) are useful in identifying tightly linked DNA markers. Most NILs have been developed by introgression. Introgression consists of repeatedly backcrossing a line carrying a gene of interest to a cultivated line chosen because of its otherwise favorable properties. After each cross, progeny are selected on the basis of the phenotype of the target gene. After several generations, the genome of the selected individuals consists almost totally of the recurrent parent except the region around the gene introgressed. However, a small segment of

foreign DNA flanking the target gene will remain. It is predicted that the target gene will be flanked by an introgressed segment extending approximately 5 cM in both directions after 20 generations (Hanson, 1959; Stam and Zeven, 1981).

The products of introgression are a pair of NILs which are identical except for a region near the target gene. If the source of the gene and the recurrent parent are sufficiently polymorphic with respect to one another, the introgressed segment can be used as a target to determine whether an RFLP marker is located near the gene of interest. Markers that are located outside the segment will exhibit identical band patterns between the NILs, while markers located inside the segment may exhibit one or more polymorphisms. It is very important that the source and the recurrent lines be divergent with respect to each other at the DNA level. If not, the introgressed segment might be missed.

Genes controlling qualitative traits can be mapped using NILs. This approach would not be suitable to map QTLs, however. Brewbaker (1995) developed a set of NILs on the same genetic background of inbred Hi27, including 120 morphological markers. This set of NILs was used to intimate the linkage relationship for maize mosaic virus

resistance gene *Mv*; this gene was suggested to be linked with two morphological markers, lg_2 and na_1, on chromosome 3 (Brewbaker, 1974). Young *et al.*, (1988) mapped two RFLP markers tightly linked to the *Tm-2a* locus in tomato using NILs. Tight linkage of the markers with *Tm-2a* was verified in a segregating F_2 population.

Bentolila *et al.* (1991) used NILs to map the *Ht1* locus in maize. It was accomplished by the use of four pairs of near isogenic lines (NILs: B73, A619, W153R, and CM105), each differing by the presence or the absence of the gene *Ht1*. Six markers exhibited an RFLP for at least one pair of NILs. Presumptive linkage was further tested by analyzing the segregation of five of the six markers (one was monomorphic in the cross studied) and resistance to *H. turcicum* race 1 on 95 F2 individuals from the cross DF20 X LH146Ht. The results indicated a tight linkage between one of the DNA markers, *umc150b*, and the *Ht1* gene. Using NILs in soybean, the *R* and *Lf1* genes (Muehlbauer *et al.*, 1991), five *Rps* loci and a *Rj2* locus were mapped with RFLP markers (Diers *et al.*, 1992).

1.4. The Pooled-DNA Analysis in RFLP Mapping

A new approach, termed pooled-DNA analysis, was developed by Michelmore *et al*. (1991). The advantages of this approach include less effort and low cost. The principle of this approach is opposite to the near-isogenic lines. The underlying assumption is that individuals from one type of genotype (susceptible or resistant to a disease in a particular case) are identical for a genomic region (a disease resistance gene), but arbitrary at all unlinked regions. The method requires preparing one (susceptible) or two (susceptible and resistant) pooled-DNA samples from a segregating population from a single cross (F_2 or RILs). The recommended pool size for the first screening is about 25 individuals. The more individuals used, the closer the marker identified will be to the target gene, but the risk of skipping the target gene also increases when the markers 20 cM apart are used for screening. For RFLP markers, as a general rule, the individuals selected for the pooled-DNA sample should be homozygous for the target gene. With an F_2 population, if the resistance gene is dominant, only the susceptible individuals could be used for a pooled-DNA sample.

RILs will be homozygous for the target alleles (e.g. resistant and susceptible), thus pooled-DNA samples from both phenotypes could be used. The approach involves identifying RFLP polymorphisms between two parents and establishing the identity of susceptible parent and susceptible pool and of resistant parent and resistant pool. For randomly amplified polymorphic DNA (RAPD) markers, it involves identifying polymorphisms between two pools containing individuals from a segregating population. The two pools are different with respect to a particular trait or genomic region and seemingly heterozygous with respect to all other regions. With the dominant nature of the RAPD markers, the absence of the band in the susceptible pool compared with the resistant pool would be observed where the resistance gene is located.

Michelmore *et al.* (1991) were able to define a 25 cM RAPD marker window, on either side of the locus responsible for downy mildew resistance in lettuce. McMullen *et al.* (1995) identified three genes, *ws1, ws2,* and *ws3*, conferring resistance to wheat streak mosaic virus in maize using a pooled-DNA approach with RFLP markers.

Churchill *et al.* (1993) proposed a pooled-sample approach to construct high-resolution genetic maps for gene cloning. Two requirements need to be met for this strategy:

1) the existence of an easily selectable target locus; 2) the ability to produce a large segregating population. The method was based on the fact that when mapping many markers in a small segment of a chromosome, very few individuals contain chromosomes with a crossover in the region of interest and thus most individuals provide little useful information. By pooling individuals for analysis, the effort required to construct a high-resolution map can be reduced manyfold.

The steps for using this pooled-mapping technique in a segregation population (F_2) are as follows: 1) identify those individuals by phenotype that are homozygous (usually homozygous recessive) for the target gene; 2) divide these individuals into a series of pools and extract DNA from each pool using approximately equal amounts of tissue from each individual; and 3) probe the pooled DNA with clones known to be located in the vicinity of the gene. The proportion of pools containing at least one crossover event is recorded, and the resulting data are used to construct a high-resolution map. The pool size is determined by the local density of markers around the target locus. The higher the marker density, the larger an optimum pool size is. This approach was demonstrated by the high resolution mapping of

a region on chromosome 5 of tomato that contains a gene, *rin,* regulating fruit ripening (Churchill *et al.*, 1993).

1.5. Statistical Analysis for Mapping Quantitative Trait Loci

The underlying assumption of using marker loci to detect QTLs is that linkage disequilibrium exists between alleles at the marker locus and alleles at the linked QTL. Linkage disequilibrium can be defined as the non-random association of alleles at different loci in a population due to a number of factors including selection, genetic drift and physical linkage (Tanksley, 1993). In primary segregating generations (eg. F_2, F_3 or backcross populations), the predominant cause of linkage disequilibrium is physical linkage of loci. This has formed the basis for classical linkage mapping for the past century. The ability to detect a QTL with an RFLP marker is a function of the magnitude of the QTL's effect on the trait, the size of the population being studied, and the recombination frequency between the marker and the QTL (Tanksley *et al.*, 1989).

There are two principal statistical approaches for determining whether a QTL is linked to an RFLP marker, and

both share the same basic principle. It is based on partitioning the population into different genotypic classes based on genotypes at the marker locus. Correlative statistics are used to determine whether the individuals of one genotype differ significantly compared with individuals of other genotype(s) with respect to the trait being measured (Tanksley, 1993). If the phenotypes differ significantly, it is interpreted that a gene affecting the trait is linked to the RFLP locus used to subdivide the population. It is not possible to determine whether the effect detected with the RFLP locus is due to one or more linked genes affecting the trait (Thompson, 1975; Tanksley, 1993) and the term RFLP locus can be loosely defined to include many gene loci (Beavis *et al.*, 1994)

1.5.1. Normal Frequency Curve Method

A normal frequency curve is commonly used to describe biological data. The two parameters of normal distribution are the mean and standard deviation. The mean determines the position of the curve on the horizontal axis. The standard deviation determines the amount of spread or dispersion among the variates. The formula for describing a normal frequency curve is

$$f = N/(\sigma * sqrt(2*pi)) * e^{-(y-\mu)^2/2\sigma^2}$$

where f is the frequency of occurrence of any given variate, y is any given variate, N is the number of variates in the population, μ is the population mean, and σ is the population standard deviation. From the formula, the normal frequency curve can be plotted by the calculation of mean and standard deviation.

Brewbaker (1993) developed a method employing a normal distribution curve to predict the number of QTLs. The parental means and variances are used to predict the distribution of segregating progeny based on monogenic, digenic and polygenic models. The expected distribution curve is compared with the experimental distribution curve. Goodness of fit for observed data to the expected is determined by chi-square and least-square estimates of deviation from regression. This method is powerful in analyzing monogenic and digenic models for a segregating population, including 1:1, 1:2:1, 3:1, and 1:1:1:1 ratio, with incorporated graphs that immediately reflect changes in means and variance. Moon (1995) used this method to analyze 20 quantitative traits in 10 RIL populations. He concluded that 13 traits were controlled by a single gene; six traits

fitted the digenic model; and one trait was governed by polygenes.

1.5.2. Single Factor Analysis of Variance

The simplest approach for detecting QTLs is to analyze the data using one RFLP marker at a time, which is known as a single factor analysis of variance (SFAOV) (Soller *et al.*, 1976; Tanksley *et al.*, 1982; Edwards *et al.*, 1987). Phenotypic means for the progeny in each marker class are compared. Significant F-values may indicate linkage of the marker and the trait of interest. Essentially this amounts to linear regression of phenotype on genotype by means of one way analysis of variance, under the assumption of normally-distributed residual environmental variance (Lander and Botstein, 1989).

In the linear model, the dependent variable is a linear function of the independent variable

$$Y = b_1X_1 + b_2X_2 + e$$

where Y is the dependent variable (a trait), X_1 and X_2 are the independent variables (two types of genotypes), b_1 and b_2 are the respective regression coefficients, and e is the

residual of *Y* not explained by the effects included in the model.

The disadvantages of the single factor analysis include: 1) its phenotypic effect may be underestimated due to recombination between the QTL and the marker; 2) the approach does not define the likely position of the QTL. In particular, it cannot distinguish between tight linkage to a QTL with small effect and loose linkage to a QTL with large effect; 3) and the method cannot tell whether the markers are associated with one or more QTLs (Edwards *et al.*, 1987; Lander and Botstein, 1989).

1.5.3. Interval Mapping

The disadvantages of single factor analysis are overcome by an approach reported by Lander and Botstein (1989), interval mapping using LOD (log of odds) score. Interval mapping measures the effect of each genome segment located between pairs of markers rather than the effects associated with an individual marker. This approach is based on the assumption of no double crossovers between the markers. The LOD score used to declare the presence of a QTL is the Log_{10} of the odds ratio. The odds ratio is the probability that the data would have arisen assuming the

presence of a QTL divided by the probability that it would arise assuming the absence of a QTL. A program MAPMAKER/QTL (Lander and Botstein, 1989; Lincoln *et al.*, 1993) was designed to map QTL using the interval mapping technique. The threshold (T) of the LOD score for the declaration of a QTL can be calculated according to the formula given by Lander and Botstein (1989):

$$T = 1/2\ (\mathrm{Log}_{10}e)\,(Z_{a/m})^2$$

where

a = the chosen level of significance

m = the number of interval markers tested

Z = standardized normal variate

The appropriate threshold depends on the size of the genome and the density of markers genotyped. Generally, the LOD score threshold varies from 2-3 for the F_2 population and 5-6 for backcross and RIL populations. The threshold is higher in a dense map compared to a sparse map.

Paterson *et al.* (1988) demonstrated the effectiveness of the LOD method in an interspecific cross of tomato (*L. esculentum* X *L. chmielewskii*). They used a saturated linkage map (a marker every 20 cM) and mapped six QTLs

associated with fruit mass, four QTLs affecting the concentration of soluble solids, and five QTLs associated with fruit pH at a LOD score of 2.4. These QTLs accounted for 44% to 58% of the phenotypic variation in these traits. Wang *et al*. (1994) studied the genetic basis of complete and partial resistance to blast disease in a RIL population derived from two rice cultivars, Moroberekan and CO39. They mapped two dominant loci associated with qualitative resistance to five isolates of the fungus, and ten QTLs affecting partial resistance at a LOD score of 6.0.

Although interval mapping increases the efficiency of QTL mapping, a large population size may still be required to map the QTL affecting the phenotype with small magnitude. Lander and Botstein (1989) suggested a method to increase the power of QTL mapping, which is selective genotyping of the upper and lower five percent of the quantitative trait distribution. This method can significantly increase the efficiency of QTL mapping and reduce the time and cost of genotyping the markers.

CHAPTER 2

MATERIALS AND MATHODS

2.1. Plant Material

A total of 129 recombinant inbred lines (RILs) were generated from the cross, Hi31 X Ki14, by Moon (1995). Hi31 is a B68 conversion, dent corn, out of Iowa Stiff Stalk Synthetic, and Ki14 is a Thai flint inbred, out of Suwan 1. The cross was made in 1987 at Waimanalo, Hawaii. Two hundred F2 seeds were randomly selected and planted by Moon (1995) in the spring of 1990. Each plant was self-pollinated without selection and harvested for the next generation (F3). Seeds from each self-pollinated ear were planted ear to row of 5 m long and 0.75 m spacing between the rows. Two plants of each row were randomly selected and self pollinated. The self-pollinated ear on the first plant in the row, when possible, was harvested to advance the line to the next generation. This single seed descent (SSD) procedure was repeated to the F7. The lines (ears) in the F7 generation were planted in an ear to row configuration in the winter of 1992 and harvested in the spring of 1993. Ten

plants in each line were sib-pollinated to give an adequate amount of seed for future experiments.

2.2. RFLP Analysis

2.2.1. Preparation of Plant Materials for DNA Extraction

The two parental lines (Hi31 and Ki14) and 123 RILs were planted at Columbia, Missouri for DNA extraction. Seedlings were harvested three weeks after planting. The samples were treated with liquid nitrogen and lyophilized for 5 days. The lyophilized samples were ground to a fine powder and stored at 4^0C.

2.2.2. DNA Extraction

Total genomic DNA was isolated from the seedlings according to the CTAB procedure described by Saghai-Maroof *et al.* (1984). Approximately 0.3g of powdered leaf tissue was incubated in 8 ml CTAB (Hexadecyl tri-Methylammonium Bromide) extraction buffer (1% CTAB, 100 mM Tris pH 7.5, 0.7 M NaCl, 10 mM NaEDTA, and 1% B-mercaptoethanol) at 65^0C for 30-60 minutes with occasional mixing to denature protein. After incubation, the sample was extracted with 4.5 ml

chloroform:octanol (24:1), and was mixed by inversion to form an emulsion. This emulsion was centrifuged for 10 minutes at 1800 g in a table top centrifuge (Beckman Model TJ-6 centrifuge) for 10 minutes at room temperature. After centrifugation the aqueous phase was separated, and 6 ml cold isopropanol was added and mixed by gentle tube inversions to precipitate the DNA (total nucleic acid). The precipitated DNA was removed with a glass hook, and transferred to 5 ml plastic tubes containing 1 ml of wash buffer (76% ethanol and 0.2 M NaOAc) for 20 minutes. The DNA was dipped in a rinse buffer (76% ethanol and 10 mM NH_4OAc) for 5 seconds. The DNA was resuspended in 0.5 ml TE (10 mM Tris-HCl pH 7.4 and 1 mM EDTA) overnight at 4^0C with gentle rocking. The solution was centrifuged for 10-15 minutes at 12000 rpm in a table top centrifuge (Eppendorf model 5415C) and the supernatant transferred to a clean 1.5 ml microfuge tube. DNA concentration was determined using the Beckman DU-65 Spectrophotometer wave length 260 nm/280 nm ratio. Finally, the DNA was diluted to a concentration of 0.5 $\mu g/\mu l$ in TE and was stored at -20^0C.

2.2.3. Restriction Digestion

A pooled-DNA sample from 28 MMV highly susceptible RILs was prepared for the pooled-DNA mapping aproach and also to verify the probe-enzyme combinations revealing polymorphism between parents Hi31 and Ki14. Genomic DNA samples (10 μg) were digested with 3 units of restriction enzyme/μg DNA in a total volume of 300 μl. The eight restriction enzymes used were *Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Sac*I, and *Xba*I (New Englang Biolabs). Restriction digestion was carried out for 4 hours in a 37^{0}C incubator. The reaction was stopped by adding 16 μl of 5 M NaCl. DNA was precipitated by adding 750 μl of ethanol and was kept at -80^{0}C for 30 minutes. DNA was then centrifuged, dried overnight, and resuspended in 40 μl TE, which allowed DNA to be loaded on agarose gel at a concentration of 10 μg/lane on an agarose gel.

2.2.4. Agarose Gel Electrophoresis

The digested genomic DNA samples were electrophoresed on 0.8% agarose gel, in 1X RBE running buffer (40 mM Tris-Acetate, pH 7.3, 2 mM Na_2EDTA, 0.02 mM NaOAC, 32 mM glacial acetate acid). Electrophoresis was carried out for

12-14 hours at 25 mA until the tracking dye (bromphenol blue) had migrated about 3/4 of the distance. Gel dimensions were 20 cm X 25 cm, which allowed four sets of combs with 30 wells to be used on a single gel. After electrophoresis, the gels were stained with ethidium bromide (1 μg/ml) for 20 minutes to permit visualization of the DNA. The gels were then rinsed in dH_2O for 20 minutes, slid onto a UV transilluminator, and photographed.

2.2.5. Southern Transfer

After electrophoresis, the gels were prepared for southern transfer in 1 liter volume of solution for each of the two following steps: 1) the DNA fragments in the gel were denatured by soaking the gel twice in 0.4 M NaOH, 0.6 M NaCl for 30 minutes; 2) neutralized in 0.5 M Tris-Acetate, 1.5 M NaCl, pH 7.5 for 30 minutes.

The DNA was transferred to nylon membranes (MSI Magnagraph, Fisher Scientific) using a method modified after Southern (1983). A plastic grid was placed in a shallow tray to allow transfer buffer (25 mM $NaPO_4$, pH 6.5) access to the center of the sponge. A thick sponge (6.25 cm X 7.5 cm) was placed on the center of the plastic grid and soaked thoroughly in the transfer buffer. Whatman 3M

chromatography paper and nylon membrane were cut to the same gel size and wetted in the transfer buffer. After two sheets of Whatman 3M paper were placed on top of sponge, the gel was placed onto Whatman sheets on the sponge. A labeled piece of membrane was placed on the gel with the label-side down to identify the transfer side of membrane, and a glass rod was used to roll out air bubbles. Two more sheets of wetted Whatman 3M paper were placed on the membrane, and a 7.5 cm stack of paper towels was placed on top of the Whatman 3M paper. More transfer buffer was added to the tray so that the buffer level remained high during blotting process. The transfer process was left overnight, and then the paper towels were carefully removed after the stack had absorbed 5 - 7.5 cm of transfer buffer. The membranes were briefly washed in 2 x SSC , dried between clean Whatman filter paper at room temperature, and baked at 80^0C for 2 hours. Membranes were then UV-cross-linked with a Stratalinker according to the manufacturer's recommendations (Stratagene, San Diego, California). The membranes were ready for hybridization or stored in a plastic bag at 4^0C.

2.2.6. Probe Labelling

A set of 163 maize RFLP probes throughout ten chromosomes were selected based on the maize RFLP linkage map (Coe, 1993) to screen the parental blots for polymorphism. These RFLP probes were public sets from the University of Missouri at Columbia, MO (umc), Brookhaven National Laboratory, Long Island, NY (bnl), California State University at Hayward, CA (csu), Native Plants Inc., Salt Lake City, UT (npi), and Pioneer Hi-Bred International, Inc., Johnson, IA (php).

The probe DNA insert from the cloned plasmid vector was purified from the low-melt agarose after restriction digestion and electrophoresis. Approximately 50 nanograms of insert DNA were used in the radioactive labelling reaction. The incorporation of radioactive ^{32}P-dCTP was done by the random priming method (Feinberg and Vogelstein, 1983). First, 10 μl of 5 ng/μl probe insert DNA and 25 μl deionized distilled water (ddH_2O) were added to a 500 μl microfuge tube. The DNA was denatured by heating at 95^{0}C for 5 - 7 minutes, and then was quenched on ice. A bulk reaction mix of OLB (0.446 M Tris-MgCl, 0.225 M random primer hexamers, and 0.223 M Hepes, pH 6.6), bovine serum albumin (BSA), dCTP, and Klenow was prepared just before use

and 15 μl aliquote placed into each tube containing DNA. The DNA insert was radiolabeled with 50 μCi ^{32}P-dCTP (New England Nucleotide, DuPont). The reaction was carried at room temperature for 4 hours. This radiolabled probe was separated from unincorporated ^{32}P-dCTP on Pharmacia Nick-Column. The purified radiolabeled probe was denatured by heating at 95^{0}C for 5 - 7 minutes, and then was quenched on ice for hybridization.

2.2.7. Hybridization

The membranes were pre-hybridized (5 X SSC, 2 X Denhardt's, 50 mM Tris-HCl, pH 8.0, 5 mM Na_2EDTA, 0.5% sarcosine, 1.0 ml of 10 mg/ml boiled salmon sperm) in a 65^0 C water bath for at least 3 hours. The prehybridization solution was then squeezed out. The denatured probe was added with 4 ml hybridization solution (5 X SSC, 2 X Denhardt's, 50 mM Tris-HCl, pH 8.0, 5 mM Na_2EDTA, 0.5% sarcosine, 10% dextran sulphate, 1.0 ml of 10 mg/ml boiled salmon sperm) per 250 cm^2 blot. The hybridization was carried out for 24 hours in a 65^{0}C oven, slowly rocking. To remove the excess probe, the membranes were washed three times for 5, 10, and 30 minutes in a low salt stringency solution (2 X SSC, 0.1% SDS) at room temperature, then

another two times in high salt stringency solution (0.2 X SSC, 0.1% SDS) at 65^{0}C. The membranes then were exposed to X-ray film with an intensifying screen at -80^{0}C for 1 to 6 days depending on the intensity of the signal. Autoradiographs were obtained by developing films in a Kodak X-OMAT M20 Processor.

The membranes were washed in 0.1X SSC, 0.1% SDS for 10 minutes, stripped in 0.1N NaOH, 0.2% SDS for 4 minutes, and neutralized in 0.2 M Tris-7.5, 0.1 X SSC, 0.2% SDS for 20 minutes. The membranes were either returned to the prehybridization solution for further hybridization with other probes, or kept for reuse in plastic bags at 4^{0}C to retard dessication.

2.3. Linkage Analysis

The polymorphic RFLP loci were mapped using MAPMAKER version 3.0 (Lincoln *et al.*, 1993). Linkage groups were based on the UMC 1993 maize RFLP map (Coe, 1993), but marker order and distances were obtained using the RILs algorithm in the MAPMAKER program, based on the segregation data of the F_7 RILs. To determine the associations between molecular markers and the MMV resistance, the single factor analysis of variance (SFAOV) was conducted using the PROC

GLM procedure in SAS (SAS Institute, 1989). MAPMAKER/QTL version 1.1 (Lincoln *et al.*, 1993) was used to identify putative loci affecting MMV resistance based on point and interval analysis. Results from these two analytical approaches were compared.

CHAPTER 3

RFLP MAPPING OF A MAJOR GENE CONFERRING RESISTANCE TO MAIZE MOSAIC VIRUS

Abstract

RFLP markers were used to investigate host plant resistance to maize mosaic virus (MMV) in 117 maize recombinant inbred lines (RILs), derived from the cross of Hi31 (a B68 conversion) and Ki14 (a Thai inbred). Ninety-one RILs were evaluated for MMV resistance in a disease nursery in Hawaii in the summer of 1994. Twenty-eight highly susceptible RILs were chosen for the pooled-sampling approach. As initial evidence from the pooled-sample, RFLP probes on chromosome 3 near the centromere were biased to the susceptible parent allele. The phenotypic data of RILs were analyzed with 127 restriction fragment length polymorphism (RFLP) loci. Loci on chromosome 3 near the centromere showed the largest effects, indicating that a major MMV resistance gene, previously named *mv*, was located in this region. This gene is present in the resistant parent Hi31 and apparently traces back to the Argentine parent used in conferring common rust resistance into B14.

We conclude that resistance to MMV in B68 and Caribbean flints involves a major gene *mv* on chromosome 3, 4 cM and 5.6 cM from RFLP markers *umc*102 and *php*20508, respectively.

3.1. Introduction

Maize mosaic virus (MMV) causes a major disease of maize (*Zea mays L.*) in the lowlands of Hawaii and many tropical and sub-tropical countries (Brewbaker, 1981). The virus is transmitted by the leafhopper *Peregrinus maidis* (Ashmead) in a persistent manner (Carter, 1941), and the disease occurs with this widely distributed vector throughout humid tropical and sub-tropical regions (Herold, 1972). It was recognized as a serious disease of maize in Hawaii as early as 1914 (Kunkel, 1921). Symptoms can be readily recognized as dwarfing internodes and husks, high-contrast chlorotic stripes along leaf veins, sheaths, stalks and husks (Herold, 1972). Symptoms are especially severe on sweet and supersweet corn when planted throughout the year for continuous marketing (Brewbaker, 1981).

Although maize mosaic is no longer as damaging as it was before the 1970s due to effective conversion to the resistance gene, severe yield losses have been reported for this disease. Furthermore, an entire field can be dwarfed

below 50 cm in height with no kernels produced under severe epiphytotics (Brewbaker, 1979). Almost all temperate hybrids are fully susceptible to MMV. No major U.S. hybrids have shown resistance in Hawaii. A resistance gene was discovered from lines of Hawaiian Sugar, tracing back to a Caribbean flint (Brewbaker and Aquilizan, 1965). This gene was introduced by direct backcrossing into more than 100 inbreds and cultivars and 130 genetic stocks (Brewbaker, 1974).

Resistance to MMV was first recognized clearly in Cuban Flint materials imported into Hawaii around 1910. The genetic basis for mosaic resistance has been studied through quantitative genetic analysis (Brewbaker and Aquilizan, 1965, Brewbaker 1981). Brewbaker and Aquilizan (1965) concluded that resistance to MMV from the Caribbean materials is monogenic with co-dominant alleles. The MMV resistance is expressed as a very high level tolerance of the virus. The heterozygotes showed partial resistance under severe epiphytotics (Brewbaker, 1981). Brewbaker (1974) suggested that this resistance gene, designated *Mv*, was linked to two morphological markers *na*1 and *lg*2 on chromosome 3.

Several viral disease resistance genes have been mapped in maize using RFLP markers (McMullen *et al*. 1989, 1991,

1994; Kyetere, 1995). The recombinant inbred lines (RILs) have been used for constructing molecular maps in plants (Burr *et al.* 1989; Reiter *et al.*, 1992) and mapping disease resistance genes (Wang *et al.* 1994; Kyetere *et al.*, 1995). The advantage of RILs in genetic study has been reviewed by Burr *et al.* (1989). A pooled-DNA approach was well developed for mapping disease resistance genes and quantitative trait loci (QTL) (Michelmore *et al.*, 1991; McMullen *et al.*, 1994; Darvasi and Soller, 1994). The objective of this study was to locate the MMV resistance gene on the RFLP map of maize.

3.2. Materials and Methods

Procedures for RFLP and statistical analysis were described in detail in chapter 2.

3.2.1. Tests for Resistance to MMV

Ninety-six RILs were planted for MMV resistance evaluation in a lattice design in the winter of 1993 at Waimanalo, Hawaii. Ninety-one RILs were grown in a randomized complete block design in the same screening site

in the summer of 1994. Twenty sub-lines each were grown of resistant parent Hi31 and susceptible parent Ki14. Two trials with two replications each were evaluated for the disease under a natural infection in a field where susceptible corn was planted successively for a year to increase the virus and vector populations. Test entries were planted in short row plots of 5 m in length and 0.75 m row spacing. Two rows of susceptible sweet corn (White Knight) were planted every eight test entries, and three rows were planted around the blocks to enhance the natural inoculum. The first 10 plants from each line were rated using a 1-9 scale as indicated below:

1 = No apparent symptom.

2 = Top two or three leaves mottled; no stunting.

3 = Entire plant above the ear mottled and/or discolored; no evident stunting.

4 = Chlorosis and/or discoloration above the ear; some stunting.

5 = Plant above the ear discolored; plants stunted and ear reduced in size.

6 = Upper three-fourths of plant chlorotic and/or discolored; plants stunted and ear reduced in size.

7 = Entire plant discolored and stunted; small ear.

8 = Entire plant discolored and stunted; no ear produced.

9 = Plant completely collapsed; no ear.

3.3. Results

3.3.1. Disease Resistance Test

After screening the RIL population for MMV resistance in the spring and summer of 1994, 50 RILs were classified as susceptible with every plant or more than 90% of the plants infected (Appendix Table 1). Thirty-nine RILs were classified as resistant (symptomless for at least one replication). The segregation ratio fitted the expected 1:1 segregation ratio ($x^2 = 1.36$, $P = 0.25$) from a single gene controlling MMV resistance (Brewbaker, 1981).

3.3.2. Screening Parental Polymorphism

Among the 163 probes tested in RFLP analysis, 114 were polymorphic between Hi31 and Ki14. The polymorphism was confirmed by pooled RILs with one or more of the eight restriction enzyme digestions. Since the RILs were derived from a tropical inbred (Ki14) and a temperate inbred (Hi31),

the overall level of polymorphism detected between the two parents was as high as 69.3%. This is higher than the 60% previously reported for temperate germplasm (Gardiner *et al*, 1993) and the 50% for tropical germplasm (Kyetere, 1995).

3.3.3. Genotyping of RILs

One hundred fourteen RFLP markers showing polymorphism between the two parents were chosen for mapping and analysis in the RILs. Since duplicated loci were exhibited from 13 probes, a total of 127 loci were genotyped for these RILs (Figure 3.1). For 19 probes, non-parental band mobilities were observed (Figure 3.2). The average frequency of non-parental alleles was 3.2%. Non-parental bands were coded as missing data.

The overall average of the parental alleles in 127 RFLP markers was slightly biased to Hi31 alleles (56.5%). Sixty five RFLP markers fit the 1:1 expected segregation ratio with an average of 50.6% Hi31 alleles. A skewed segregation was observed for 48 RFLP loci favoring Hi31 alleles (average 71.6%), and only 14 RFLP loci there were 64.5% Ki14 alleles (Figure 3.3).

Of the 117 RILs, four lines were eliminated due to too many missing data for two lines and too many heterozygous

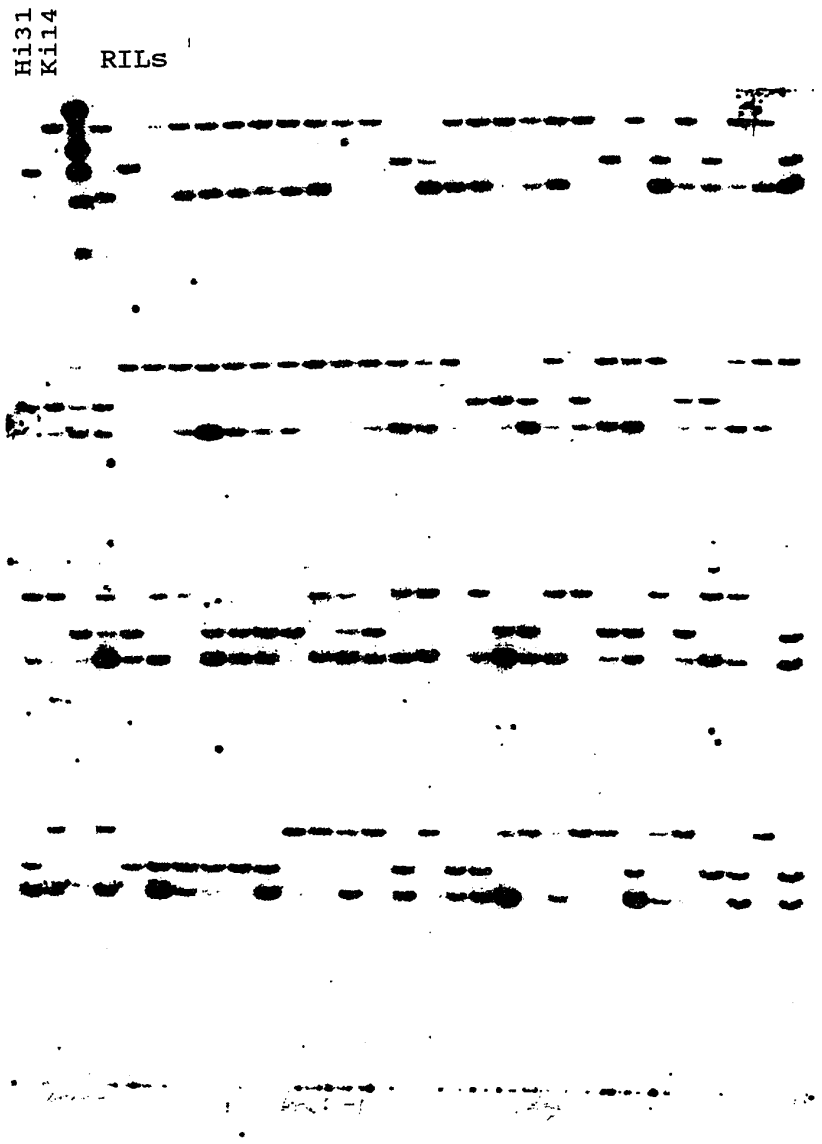
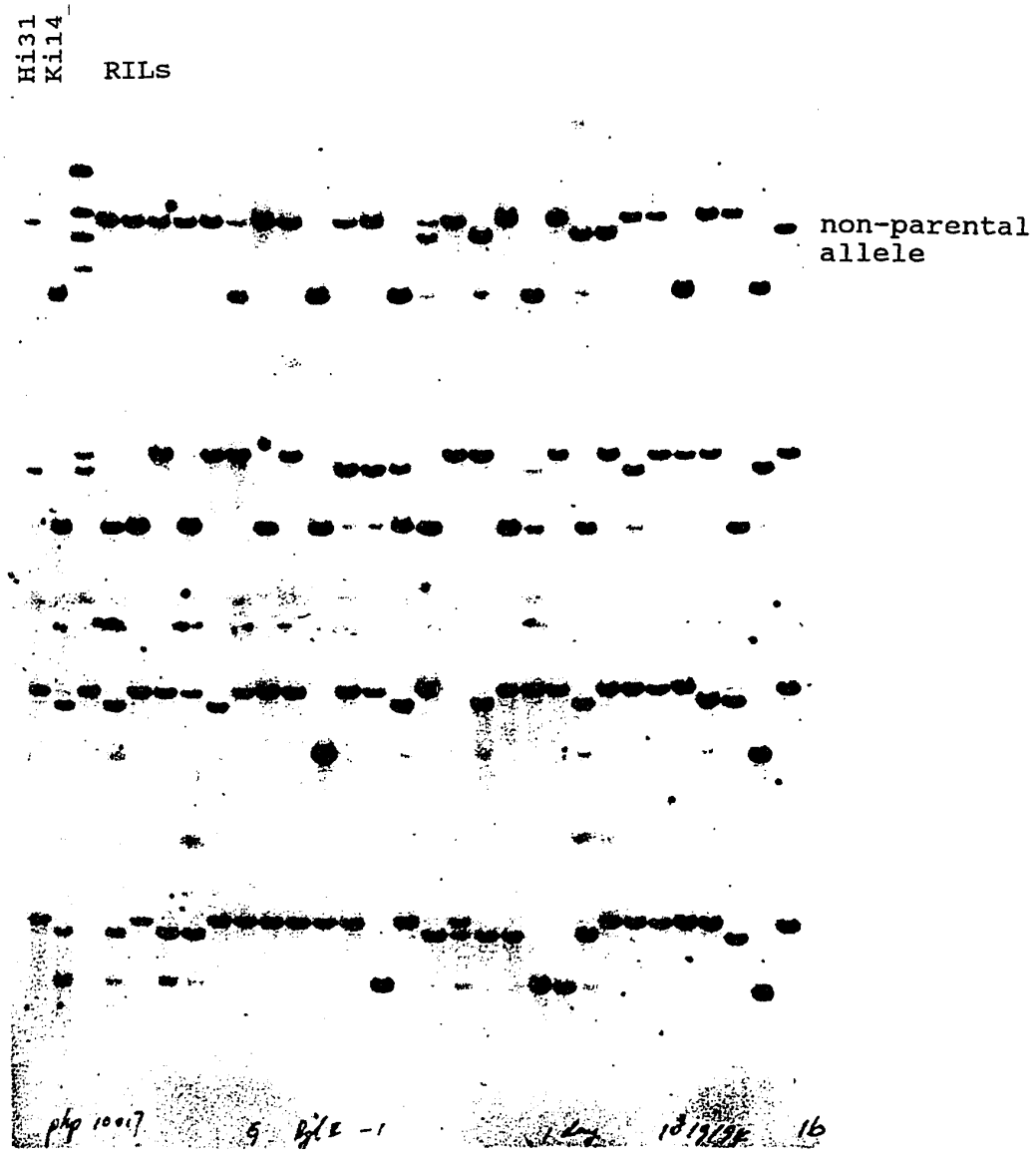


Figure. 3.1. Segregation of 117 RILs of maize (Hi31 X Ki14) for RFLP marker *umc2* and restriction enzyme *Dra*I.



Figire. 3.2. Non-parental alleles in RIL population (Hi31 X Ki14) from RFLP marker *php10017* and restriction enzyme *Bgl*II.

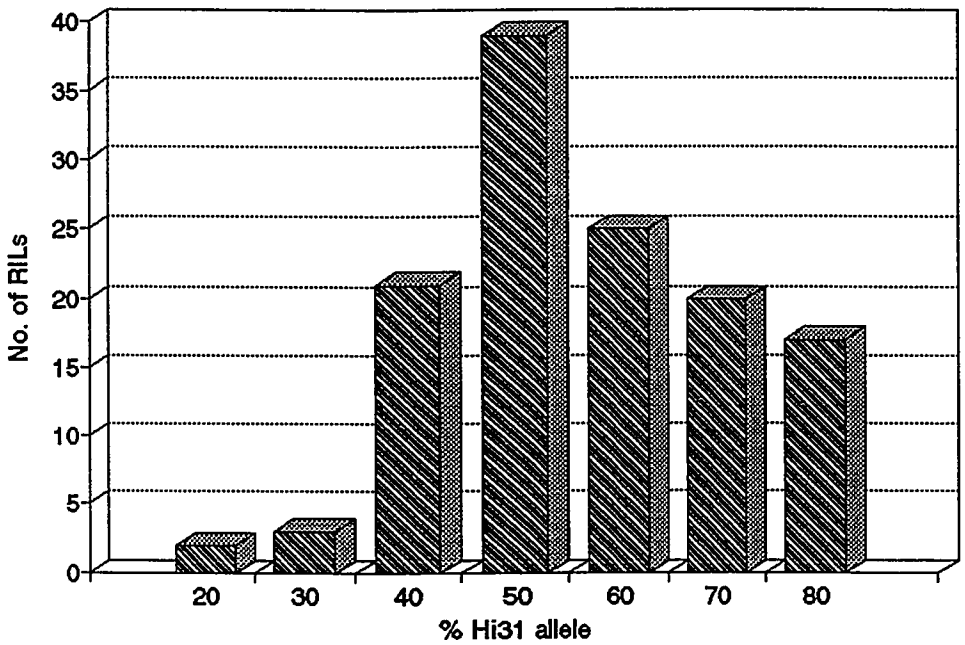


Figure 3.3. Distribution of percent Hi31 alleles for 127 RFLP markers among 113 RILs of maize (Hi31 X Ki14)

bands for the others (27 and 42 out of 127 RFLP markers, respectively). For the remaining 113 RILs, 83 RILs fit the 1:1 segregation ratio for the 127 markers ($x^2 = 0.4 < P_{0.05} = 3.84$) with a slight bias to the Hi31 alleles (54.7%). Twenty-four RILs were skewed to Hi31 alleles (68.7%) and the the remaining six RILs were skewed to the Ki14 alleles (66.3%). The overall average of the Hi31 alleles in 113 RILs was 56.5%, indicating that the RILs favored Hi31 alleles during their development (Figure 3.4).

When examining the segregation of the non-parental alleles in the RILs (Figure 3.5), the 113 RILs could be easily divided into two groups, 56 RILs with equal or less than 6 non-parental alleles and 57 RILs with more than 6 non-parental alleles. The genotypes of these two groups were analyzed with the 127 RFLP markers. In the low non-parental allele group (56 RILs), the overall average of the parental alleles fitted the 1:1 segregation ratio ($x^2 = 0.001$) with 50.3% Hi31 alleles. In the high non-parental allele group (57 RILs), however, thirty-nine RFLP markers were almost completely biased to Hi31 alleles and one marker was biased to Ki14 alleles (Table 3.1). The extremely skewed segregation represented a significant problem in constructing the RFLP map.

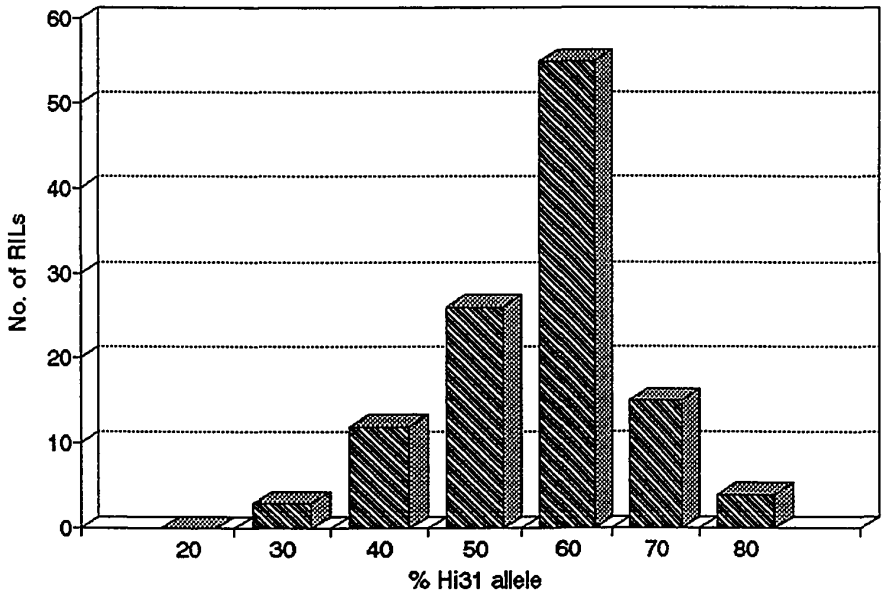


Figure 3.4. Distribution of percent Hi31 alleles for 113 RILs of maize (Hi31 X Ki14) among 127 RFLP loci

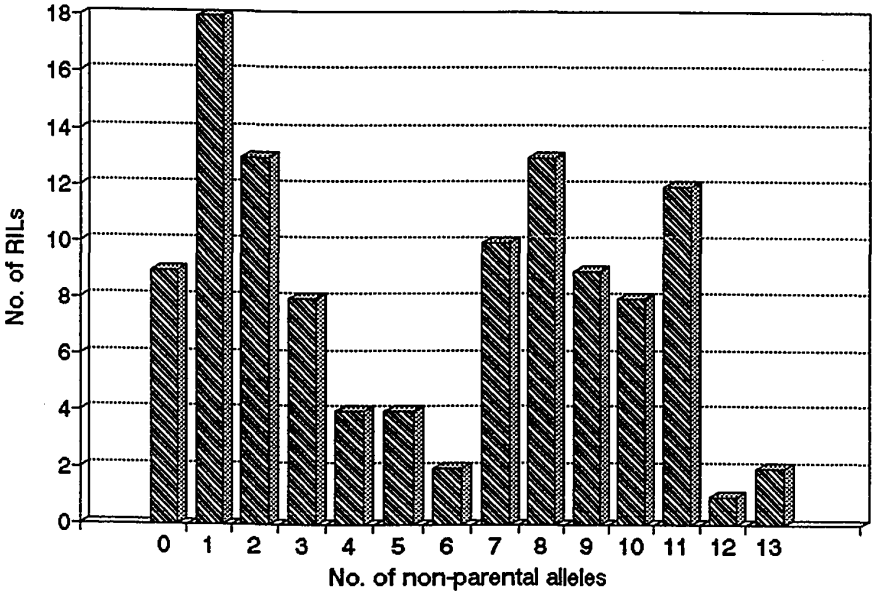


Figure 3.5. Distribution of non-parental alleles for 113 RILs of maize (Hi31 X Ki14) among 127 RFLP loci

Table 3.1. Loci showing distorted segregation in 57 RILs with high non-parental alleles.

		Number of RILs						
Locus	Chromosome	Hi31	Ki14	H	C	Missing	X^2	
npi262	1	56	0	0	0	1	56.00	**
umc167	1	26	0	0	28	3	26.00	**
umc67	1	27	0	1	29	0	27.00	**
npi238	1	26	0	0	1	30	26.00	**
bnl8.29b	1	53	0	0	0	4	53.00	**
umc53	2	26	2	0	25	4	20.57	**
npi239	2	27	0	0	29	1	27.00	**
umc131	2	54	0	0	0	3	54.00	**
umc198	2	53	0	0	0	4	53.00	**
umc198b	2	27	4	0	24	2	17.06	**
umc122	2	57	0	0	0	0	57.00	**
csu16	3	56	1	0	0	0	53.07	**
php20024	3	29	0	0	25	3	29.00	**
umc50	3	35	1	0	21	0	32.11	**
umc26	3	54	0	1	0	2	54.00	**
bnl1.297	3	57	0	0	0	0	57.00	**
umc16	3	33	0	0	0	24	33.00	**
umc63	3	31	0	0	22	4	31.00	**
php20725	4	57	0	0	0	0	57.00	**
umc200	4	57	0	0	0	0	57.00	**
umc193	4	2	25	24	3	3	19.59	**
umc156	4	57	0	0	0	0	57.00	**
umc19	4	56	0	1	0	0	56.00	**
umc15	4	28	4	0	25	0	18.00	**
bnl8.23	4	55	2	0	0	0	49.28	**
bnl6.25	5	24	3	1	29	0	16.33	**
umc72	5	27	0	0	30	0	27.00	**
umc43	5	24	0	0	33	0	24.00	**
umc132	6	54	1	0	0	2	51.07	**
umc62	6	27	2	1	27	0	21.55	**
umc134	6	57	0	0	0	0	57.00	**
bnl8.39	7	54	1	0	0	2	51.07	**
bnl16.06	7	29	0	0	28	0	29.00	**
umc103	8	51	0	1	0	5	51.00	**
umc113	9	51	5	0	0	1	37.79	**
umc190	9	54	1	0	0	2	51.07	**
bnl5.09	9	53	0	0	0	4	53.00	**
csu50	9	54	0	0	0	3	54.00	**
php20075	10	57	0	0	0	0	57.00	**
npi285	10	46	5	0	0	6	32.96	**

* H: heterozygous locus
* C: non-parental locus
* X^2: Chi square value. ** $p < 0.01$

3.3.4. Map Construction

Since the RIL data were analyzed under the assumption of a 50:50 allele distribution, a group of 65 markers was formed using the MAPMAKER group command due to the problem mentioned above. For this reason, the primary linkage groups were based on the UMC RFLP map (Coe, 1993), and did not included the secondary loci. The order and distance of those markers were derived from the RILs data obtained during this study based on MAPMAKER 3.0. Any markers more than 50 cm apart from other markers were removed from the map. Six secondary RFLP loci were placed on the map using the 'try' command. A total of 103 RFLP loci were mapped, with total length 1624.7 cM and average density 15.8 cM. Twenty-four RFLP loci were not assigned to linkage groups. No major disagreements were found with the order derived from immortalized F_2 population described by Gardiner *et al.* (1993).

3.3.5. Mapping the Maize Mosaic Virus Resistance Gene

Initial evidence for the *Mv* map position was obtained from the pooled-sampling approach as probes on chromosome 3 near the centromere were biased to the susceptible parent

allele. The SAS/GLM procedure was used to determine the correlations between RFLP markers and the MMV resistance (Table 3.2). Eight markers significantly associated with MMV resistance concentrated on chromosome 3. The marker with the highest *F* value was php20508 (F = 57.19 and LOD = 10.3), with an R^2 = 0.417, i.e., accounting for 42% of the phenotypic variation for MMV resistance. The MMV resistance gene was mapped between *umc*102 and *php*20508, 4 cM and 5.6 cM from the markers respectively. This gene accounted for 74.4% of the phenotypic variation with LOD score of 14.1 (Figures 3.6 and 3.7). This gene is present in the resistant parent Hi31 and apparently traces back to the Argentine parent used in conferring common rust resistance into B14. The results confirmed Brewbaker's suggestion from near-isogenic conversions that *mv* is linked to lg_2 and na_1 on chromosome 3 (Brewbaker, 1974). A number of RFLP probes on chromosomes 4, 7, and 9 were marginally significant for MMV resistance from the results of least squares estimates but not MAPMAKER/QTL (LOD < 6.0). Possible QTLs for MMV resistance on these chromosomes will need to be confirmed with larger populations.

Among the 39 RI lines showing resistance in the population, 34 lines had the resistant parent Hi31 allele for both markers tightly linked (*umc*102 and *php*20508), three

Table 3.2. The loci significantly associated with MMV resistance from single factor analysis of variance

Marker	Chromosome	R^2	Prob.
umc121	3	0.0615	0.0206
csu16	3	0.1983	0.0031
php20024	3	0.2409	0.0020
umc102	3	0.3202	0.0001
php20508	3	0.4169	0.0001
csu30	3	0.3768	0.0001
umc26	3	0.4014	0.0001
bnl5.37	3	0.1491	0.0003

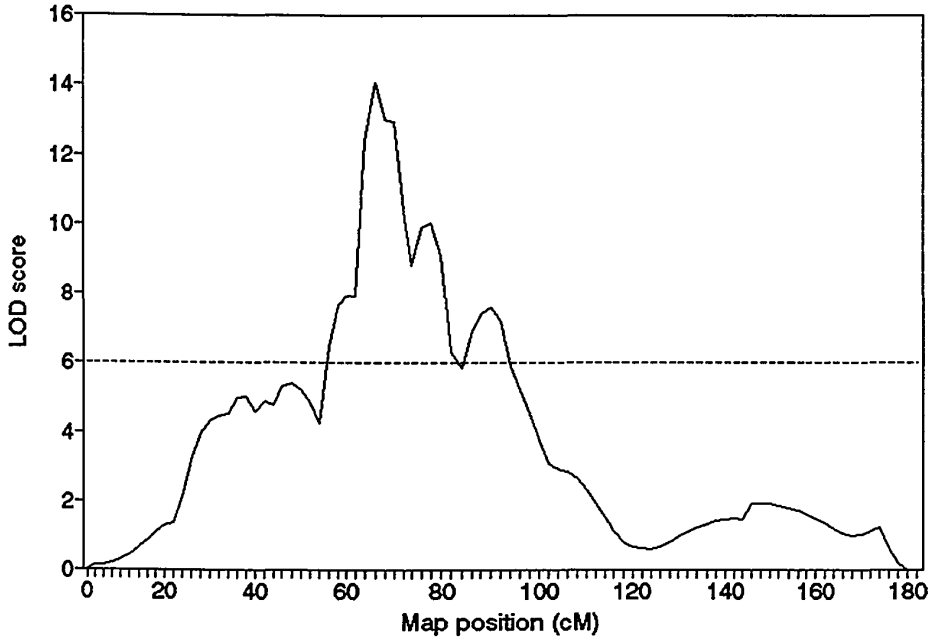


Figure 3.6. LOD score of the regions around *mv* gene on chromosome 3.

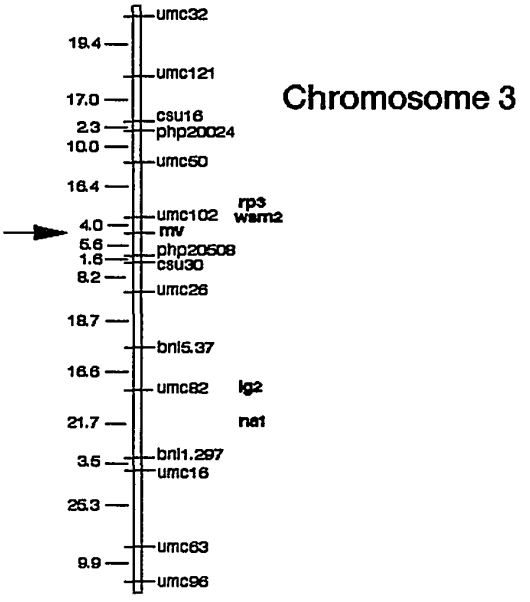


Figure 3.7. The map position of *mv* gene on chromosome 3 of maize.

lines had Hi31 allele at *umc*102 but Ki14 allele at *php*20508, two lines had Hi31 allele at *php*20508 but Ki14 allele at *umc*102. Among the 50 susceptible lines in the RIL population, 41 lines had Ki14 allele for both or either *umc* 102 and *php*20508, but nine lines had the Hi31 allele for both markers. This might be explained by a single cross-over in different generations during the development of the RILs.

3.4. Discussion

3.4.1. Disease Resistance Test

Since MMV can not be mechanically transmitted and only can be transmitted by plant hoppers, the reliability of a field test under natural infection is always subject to challenge. The RILs have considerable advantage in this situation. From the disease nursery in the spring of 1994, 46 RILs were symptomless in two replications, while 50 RILs showed susceptibility to MMV. Among these 50 RILs, 32 of them were confirmed by replication and 18 were not. Only several plants of each susceptible RIL were infected due to low disease pressure. After continuous planting of the susceptible checks in the field, 91 RILs were screened

again. This time they were under severe disease pressure. The 20 sublines from susceptible parent Ki14 showed 100% infection. Among the 50 RILs showing susceptibility, 36 RILs were confirmed by at least one replication with every plant infected and the other replication with the majority of plants infected. The other 14 RILs were confirmed by replications with a majority of the plants infected, and also confirmed by the data from the spring nursery. Thirty-nine RILs showing resistance were confirmed by at least one replication with every plant symptomless and the other replication with no or few plants infected. For diseases like MMV, RILs provide homozygosity, which allows repeated tests in different seasons and locations with the same materials.

3.4.2. Genotyping of the RILs

Despite the distorted segregation, the overall average of the parental alleles segregation of RFLP fit the 1:1 segregation ratio ($x^2 = 1.56$). Compared with the theoretical 1.56% heterozygosity of the F_7 generation (Falconer, 1989), the average 1.7% heterozygous allele was acceptable. The average of 3.2% non-parental alleles is reasonable if theoretical 98.4% homozygosity is expected for

parental inbreds of the S_6 generation. From the clear 1:1 segregation of low and high non-parental alleles, it seems that one parental line (Hi31) has higher homozygosity than the other (Ki14), but why the severely distorted segregation was concentrated on the high non-parental allele RIL group was unknown.

3.4.3. Linkage Map Construction

Forty out of 127 RFLP loci with allele segregation in a distortion ratio caused a serious problem while building the map. Only after deleting the biased alleles in 57 RILs (Table 3.1), was an acceptable map constructed. Distorted segregation were also reported in rice (McCouch *et al*, 1988; Wang *et al.*, 1994), tomato (Paterson *et al.*, 1988), barley (Graner *et al.*, 1991), and maize (Beavis and Grant, 1991; Gardiner *et al.*, 1993; Kyetere, 1995). Gardiner *et al.* reported that distortion occurred in maize chromosomes 1, 2, 3, and 5. In the present study, RILs derived from tropical and temperate germplasm showed distorted segregation for all ten chromosomes (Table 3.1). Although distortion segregation may be caused by gamete selection, inversion, and also selection(Zamir and Tadmor, 1986), no conclusion can be drawn about mechanisms for these materials.

3.4.4. MMV Resistance Gene(s)

Besides the major MMV resistance gene on chromosome 3, one region on the long arm of chromosome 9 accounted for 19% of phenotypic variation (data not shown). Checking the genotype of the linked markers, distortion segregation was observed in the markers. It is difficult to conclude if this region is a real modifier (minor gene) or the effect of the distortion segregation. A similar situation was found in a region on chromosome 4. Even though from previous studies the MMV resistance was controlled by a major gene (Brewbaker, 1974, 1981), it is always possible that some modifiers existed in the disease resistance system.

Due to the homozygosity of the RILs, the gene action of *mv* could not be determined from this study. Previous studies, however, detected the co-dominant nature of this gene (Brewbaker and Aquilizan, 1965; Brewbaker, 1981).

The MMV resistance gene *mv* was located near the centromere of chromosome 3 between RFLP markers *umc*102 and *php*20508. This map position placed *mv* close to the position *wsm*2, a dominant gene for resistance to wheat streak mosaic virus on chromosome 3 in Pa405 (McMullen *et al.*, 1994), and *rp*3, a dominant gene for resistance to *Puccinia sorghi*. Since Pa405 is susceptible to MMV, the dominant resistance

allele *Wsm*2 and co-dominant allele *Mv* should not be the same gene. It is unlikely that the fungal disease resistance gene *rp*3 and the viral disease resistance gene *mv* are allelic. Since *mv* was from an Argentine parent used to confer common rust resistance to B14, these two genes must be tightly linked. These results support McMullen's suggestion (1994) that a possible clustering of genes for resistance to maize pathogens is located in this region.

3.4.5. RFLP Marker-Assisted Backcross Breeding

The technique of backcross breeding was developed as a method to transfer target gene(s) from the donor parent to a elite inbred line as recurrent parent. The hybrid derived from a cross between the donor and recurrent parents is crossed back to the recurrent parent, and the progeny are screened for the target trait. The individuals with the target character are crossed back to the recurrent parent and the process repeated. After five or six cycles, plants are nearly identical genetically to the recurrent parent, except the region around the target gene(s) transferred. This method provides a convenient procedure for the introduction of relatively simply inherited traits into an

established line, but is ineffective for traits controlled polygenically.

The application of the RFLP technique can overcome the limitations of backcross breeding. If the target gene(s) are tagged with tightly linked RFLP markers, the plants of backcross populations can be screened at the seedling stage for the presence of the target gene(s).

There are two major advantages of RFLP marker-assisted breeding. First, the RFLP technique provides a tool for indirect selection for the traits which are difficult to screen in the field. Selection for resistance to naturally infected viruses, when no mechanical inoculation is successful, demonstrates the advantage of indirect selection with RFLP markers. With the traditional method, the screening materials must be planted in the area where the virus occurs epidemically, but the reliability of the results is still questionable. With the RFLP marker-assisted selection approach, the breeding can be carried out in any kind of environment with no field testing, and the screening for virus resistance will be done in the lab by looking for the flanking RFLP markers. The probability of a false selection is 16 in a million when two markers, each 2 cM apart from the target gene, are used.

Secondly the selection efficiency for the polygene controlled traits is dramatically increased with the RFLP marker-assisted approach. There is no suitable tool to screen for individual QTLs in a polygenic traits by traditional methods. RFLP markers that flank the target QTLs might be used effectively in the selection of these traits (Stuber, 1992).

A strategy is developing to convert *Mv* gene into 10 inbreds applying the RFLP marker-assisted approach (Brewbaker, personal communication). Inbred Hi31 or one of the resistance RILs will be used as the *Mv* gene source and crossed with 10 susceptible inbred lines (Figure 3.8). F_1 hybrids will be backcrossed with the susceptible inbreds to produce the BC_1 populations. The BC_2 populations will be produced by using recurrent parents as female and bulked pollen of 20 BC1 plants as male. The process will be repeated to obtain BC3 populations. The expected *Mv/mv* heterozygous genotype in BC_3 is 12.5%. At this stage, one hundred BC_3 plants will be genotyped with the two RFLP markers flanked to the *Mv* gene, *umc102* and *php20508*. About ten plants with *Mv/mv* genotype will be selected to backcross with the recurrent parents. The backcross process will be repeated again until BC_6. One hundred BC_6 plants will be genotyped, and about ten plants with *Mv/mv* genotype will be

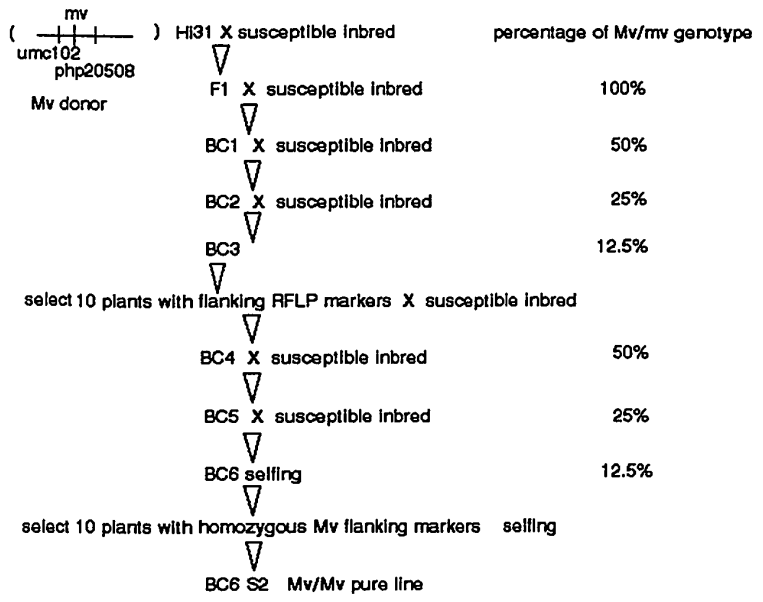


Figure 3.8. *Mv* conversion program using RFLP flags.

selfed to produce BC_6S_1 population. A 1:2:1 segregation ratio will be expected in BC_6S_1 generation. Fifty plants from BC_6S_1 will be screened with the RFLP markers to identify plants with *Mv/Mv* genotype. These plants will be selfed to produce pure lines of recurrent parents with the *Mv* gene incorporated, and the pure lines grown under virus epidemic to validate their homozygosity for resistance.

CHAPTER 4

IDENTIFICATION OF RFLP MARKERS LINKED TO A MAJOR GENE, *Sw1*, CONFERRING RESISTANCE TO *Erwinia stewartii*

Abstract

Resistance to *Erwinia stewartii* (Stewart's bacterial wilt) was studied using recombinant inbred lines (RILs) derived from a cross between Hi31 (a B68 conversion) and Ki14 (a Thai flint inbred). Seventy-one RILs and ten sub-lines of each parent were planted at Henderson, Kentucky for evaluation and were naturally infected by Stewart's bacterial wilt. The RILs were scored for wilt severity in two replications using a 1-9 scale (1=no symptom, 9=severe symptom).

The sub-lines of resistant parent Ki14 and susceptible parent Hi31 averaged 1.91 and 6.50, and ranged from 1.0 to 4.0 and 4.0 to 8.0, respectively. The F_1 hybrid, with a score of 4.08, showed intermediate resistance. The data indicated only one major dominant resistance gene involved in this population.

The RILs ranged from 1.2 to 7.9. Thirty-eight RILs were classified as resistant with an average score of less

than 4.5; thirty-three RILs were classified as susceptible. The 1:1 segregation demonstrated that a single gene controlled *E. stewartii* resistance. One hundred twenty-seven RFLP loci were analyzed with the disease data using SAS/GLM and MAPMAKER/QTL. Markers on the short arm of chromosome 1 showed the largest effects on conferring resistance, suggesting a major QTL in this region. This gene is suspected to be the previously designated *sw1*, and was mapped with LOD score 13.2 on the short arm of chromosome 1, 4 cM and 8.2 cM from RFLP marker *umc167* and *umc67*, respectively. From the results of MAPMAKER/QTL, 80.2% of the phenotypic variation for disease resistance could be explained by *sw1*.

4.1. Introduction

Stewart's bacterial wilt, caused by *Erwinia stewartii* (E. F. Smith) Dye, has been recognized as an important disease of maize (*Zea mays* L.). Catastrophic economic losses were caused by this disease in the 1930s. More recently, Stewart's wilt has occurred in the southern and northeastern corn belt of the United States (Heichel et al., 1977), Italy (Anonymous, 1983), and Canada (Anderson and Buzzell, 1986). The disease is a major concern for

international seed shipment, since the bactirium can become seedpborne.

Economically and environmentally, the most effective means of controlling Stewart's wilt is the development of resistant hybrids. Associations between the disease and other traits such as vigor and maturity were examined in early studies. Ivanoff and Riker (1936) suggested that three factors were correlated with severity of Stewart's disease: vigor, maturity, and genetic resistance. Reddy and Holbert (1928) reported no correlation of resistance with vegetative vigor or days to maturity. Wellhausen (1937) , however, observed the association of late maturity with resistance and early maturity with susceptibility.

The gene number involved in resistance has been analyzed from F_2 and backcross (BC) populations. Wellhausen (1937) concluded that two major and one minor dominant, independently inherited, supplementary genes (*Sw1, Sw2,* and *Sw3*) were involved in the resistance to Stewart's wilt. High resistance resulted from the presence of all three genes either in heterozygous or homozygous dominant conditions. Intermediate resistance could be explained by the presence of only one or any two dominant gene(s).

Smith (1971) proposed that four genes were involved in the resistance. Two major dominant genes, *Sw1* and *Sw2,* were

required for full resistance. A single major dominant gene alone conditioned intermediate resistance, such as occurred in inbred M14. At each of the other two minor dominant genes, *Sw3* and *Sw4*, resistance occurred only when the alleles were heterozygous. Parker (1980) estimated gene numbers (Wright's formula, 1921) conditioning resistance; these varied from 1.10 to 3.20 and 1.99 to 4.12 in four different families in two different years. In his studies, generation mean analysis indicated significant additive and dominance genetic effects in all families in both years. Most of the variation among families was attributable to additive gene action.

The linkage relationships of these genes are unknown. Wellhausen (1937) suggested that one of the two major genes was linked with the *pl* gene for cob color, but was not very close. Wellhausen also observed that late maturity and resistance tend to remain together in the later generation progenies. RFLPs have been effectively used to map disease resistance genes in maize (McMullen et al., 1989, 1991, 1994, Bentolila et al., 1991, Simcox et al., 1993). The objective of this study was to identify the linkage relationships of *E. stewartii* resistance gene(s) using RFLP markers.

4.2. Materials and Methods

Details of plant materials, RFLP and statistical analysis were described in chapter 2.

4.2.1. Disease Nursery

One hundred RILs were planted to evaluate blight resistance in a randomized complete block design in the summer of 1994 at Henderson, Kentucky by the Northrup King (Seeds) Company (R. Holley, unpublished). Ten sub-lines each were grown of resistant parent Ki14 and susceptible parent Hi31. The bacterium *E. stewartii* overwinters in mature corn flea beetles. In the disease nursery at Henderson, beetle populations were high and the field came under severe Stewart's wilt pressure. The first ten plants from each line in the two replications trial were rated for Stewart's wilt using a 1-9 scale as indicated below (Pataky, 1990):

1 = No symptoms.

3 = Slight chlorosis of lower leaves, 5 to 10% of the leaf area symptomatic.

5 = Chlorosis of lower and upper leaves, 21 to 30% of the leaf area symptomatic.

7 = Chlorosis, necrosis, and stunting, 41 to 50% of the leaf area symptomatic.

9 = Severe stunting and necrosis, nearly dead.

4.3. Results

The results of RFLP analysis and the construction of RFLP map were described in chapter 3.

4.3.1. Disease Resistance Test

Among the one hundred RILs planted at Henderson, twenty nine RILs did not germinate, evidently due to herbicide damage (Beacon). Seventy-one RILs were thus scored for Stewart's bacterial wilt severity in two replications. The average score of two replications from each RIL was used for analysis. The sub-lines of resistant parent Ki14 and susceptible parent Hi31 averaged 1.91 and 6.50, and ranged from 1.56 to 2.61 and 6.19 to 6.71, respectively. The F_1 hybrid, averaged 4.08, showed intermediate resistance. This was interpreted to indicate that only one of the two major resistance genes, *sw1* and *sw2* (Smith, 1971), was segregating in this population.

The average scores for RILs ranged from 1.2 to 7.9, and could easily be grouped in two clusters (Figure 4.1). Thirty-eight RILs with an average score of less than 4.5 were clasified as resistant, and thirty-three RILs with an average score higher than 4.5 as susceptible. The segregation fitted the 1:1 ratio ($x^2 = 0.35$, $p > 0.05$) expected for the single gene control.

4.3.2. Mapping *E. stewartii* Resistance Gene

Linkage analysis of RFLP loci related to *E. stewartii* resistance was conducted using single factor ANOVA. Sixteen markers were associated significantly with Stewart's wilt resistance; these were on chromosomes 1, 4, 7, and 9 (Table 4.1). The RFLP marker *umc167* on the short arm of chromosome 1 showed the highest *F* value with an $R^2 = 0.663$. Two linked markers also associated highly with resistance.

A scan of all 10 chromosomes using the MAPMAKER/QTL program revealed one peak close to the marker *umc167,* with a LOD score 13.2 (Figure 4.2). This gene was mapped 4.0 cM and 8.2 cM from RFLP markers *umc167* and *umc67*, respectively (Figure 4.3), and explained 80.1% of phenotypic variation for disease resistance in this RIL population. The map position was 38 cM apart from the p1(*umc185*) locus (Coe,

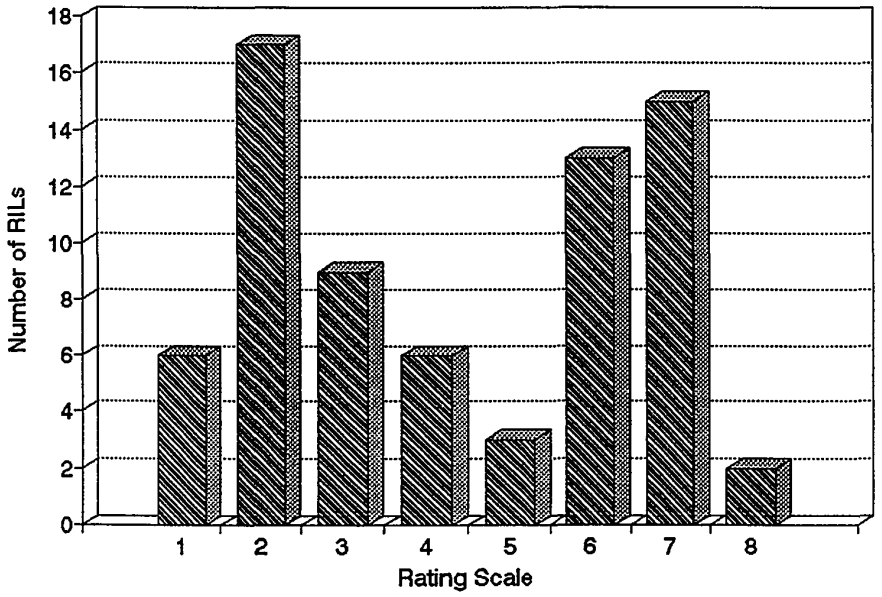


Figure 4.1. Distribution of 71 RILs of maize (Hi31 X Ki14) for reactions to *Erwinia stewartii*

Table 4.1. The loci significantly associated with *E. stewartii* resistance from single factor analysis of variance.

Marker	Chrom.	Position*	R^2	Prob.
npi262	1	84	0.2848	0.0001
umc167	1	107	0.6625	0.0001
umc67	1	114	0.2844	0.0006
bnl5.09	1	135	0.0888	0.0244
bnl8.29	1	264	0.1125	0.0389
npi287	2	60	0.1292	0.0238
umc200	4	31	0.1133	0.0367
umc156	4	90	0.3103	0.0001
umc119	4	123	0.1047	0.0478
umc72	5	40	0.0876	0.0458
csu173	5	117	0.1726	0.0330
bnl14.07	7	105	0.1551	0.0106
bnl8.39	7	115	0.2897	0.0001
bnl16.06	7	143	0.1995	0.0117
umc103	8	31	0.2704	0.0002
umc190	9	60	0.2130	0.0016
bnl7.50	9	86	0.0917	0.0416
bnl5.09	9	145	0.0956	0.0193
npi291	9	155	0.2738	0.0019
csu50	9	184	0.1840	0.0051

* The map position was based on UMC 1993 RFLP map

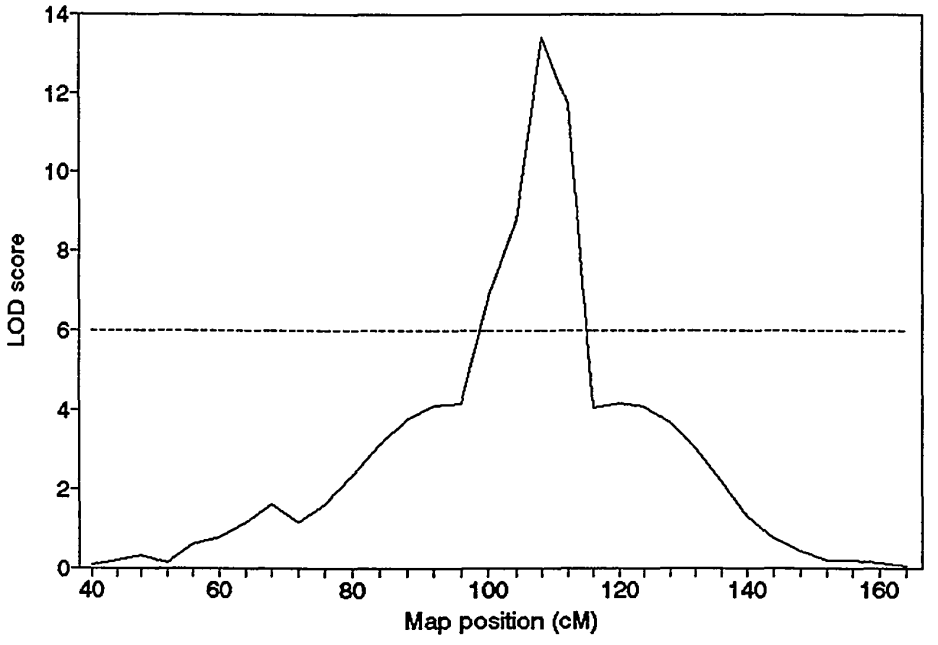


Figure 4.2. LOD score of the regions around *sw1* gene on chromosome 1.

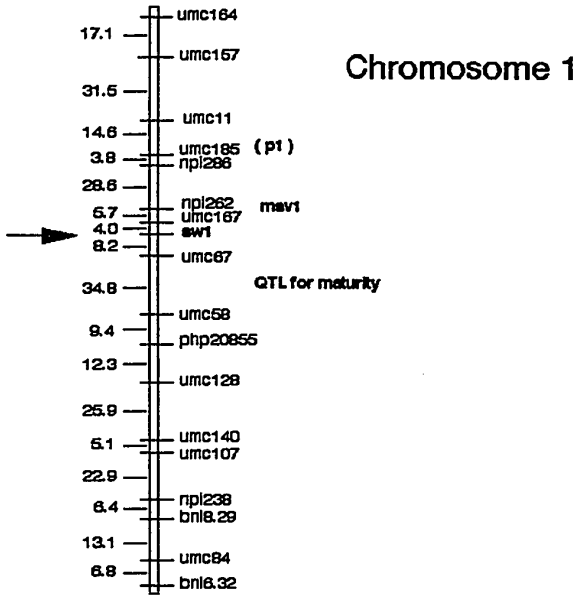


Figure 4.3. The map position of *sw1* gene on chromosome 1 of maize.

1993), and also close to one of the three QTLs for maturity (Koester *et al.*, 1993). Observations confirm the suggestion by Wellhausen (1937) that resistant allele *Sw1* was in this region on chromosome 1. We thus assume the gene segregating among the RILs derived from resistant parent Ki14 to be *Sw1*.

The only other peak formed was on the short arm of chromosome 4 with LOD 7.2, although regions on chromosome 7 and 9 displayed high R^2 values. Because of the complicated nature of the minor genes involved in this disease resistance system, we could not conclude if there were any minor genes present in the RIL population.

The resistance genotype of the RILs could be checked directly with the field data after flanking the RFLP markers with the *Sw1* allele. Of the 33 RILs classified as susceptible, 24 lines had the susceptible parent Hi31 allele for both flanked RFLP markers *umc167* and *umc67*; two lines had Hi31 allele at *umc167* but Ki14 allele at *umc67* which indicated crossovers occurred; one line had the Ki14 allele at both markers. In addition, four lines had non-parental alleles and two lines had null alleles, and these were treated as missing data. For another tightly linked marker *npi262* without non-parental allele involved, 29 susceptible RILs had Hi31 allele; two lines had Ki14 allele; the genotype data for other two lines were missing.

For the 38 RILs classified as resistant, ten lines had a resistant parent Ki14 allele at both flanked markers; seven lines had Ki14 allele at either *umc167* or *umc67*; seven lines had non-parental alleles and three lines had null alleles which were treated as missing data; eleven lines, however, had susceptible parent Hi31 allele. Since the disease nursery was under natural infection, those lines with susceptible parent allele might be escapes. When ten lines with a disease score of less than 3.2 were treated as missing data and the other line with a disease score of 3.75 was kept, the R^2 and LOD score increased significantly from 0.32 and 4.2 to 0.66 and 13.2, respectively. If ten lines were deleted randomly, the changes of R^2 and LOD score were within 1 fold.

4.4. Discussion

One of the advantages of RILs is that there is a pure line representing each genotype, instead of a single plant in a segregating population. For disease testing under natural infection, the confirmation from more than one plant is essential. As an example from this study, of the 167 plants from 10 sub-lines of the susceptible parent Hi31 in two replications, two, five, and six plants rated 2, 3, and

4, respectively, and the rest rated 5 to 8. Averages of the 10 sub-lines ranged from 6.19 to 6.73, eliminating the effect of the few plants that evidently escaped infection. The resistant parent Ki14 ranged from 1 to 4 on the basis of individual plants and 1.56 to 2.61 on the basis of sub-lines.

The susceptible parent Hi31 was derived from B14. From the studies of the 3 F_2 and 7 BC populations involving B14 as one of the parents, Smith (1971) assigned the genotype of B14A as *sw1sw2sw3sw4*, recessive at all the resistance loci. In the present study, the intermediate reaction of F_1 and the 1:1 segregation of RILs to Stewart's wilt demonstrated that only one of the two major dominant genes was present in the resistant parent Ki14. The condition of the minor genes in Ki14 is unknown. Smith (1971) suggested that the heterozygosity of two minor genes was required for resistance. If this conclusion is true, the effect of the minor gene would be eliminated, since almost all the loci in RILs are homozygous.

The linkage of morphological marker *p1* to one of the two major resistance genes confirmed the map position of *sw1* on chromosome 1 (Wellhausen, 1937). In the present study, significant regions for *E. stewartii* resistance occured also on chromosomes 4, 7, and 9 for resistance to maize mosaic

virus. It was unknown if these regions were associated with plant growth or plant vigor. Checking the genotype of the linked markers on these regions, distortion segregation was observed in a majority of the markers. It may play a role for the significance. A larger population will be required to identify the minor genes.

A gene, *msv1*, for resistance to maize streak virus was located between RFLP markers *npi262* and *umc167* on chromosome 1 (Kyetere, 1995). The map position of *sw1* thus adds another cluster of genes for resistance to maize pathogens. One other cluster is located on chromosome 3, including *rp3*, *ws2*, and *mv* (McMullen and Louie, 1989, Ming *et al.*, 1995), while a third is on the short arm of chromosome 6, with *wsm1*, *mdm1*, and *rhm1* (McMullen *et al.*, 1994). From these results of the past few years, we might expect that more disease resistance genes may be discovered in these regions.

CHAPTER 5

IDENTIFICATION OF RFLP MARKERS LINKED TO QTLs CONTROLLING RACE-NONSPECIFIC RESISTANCE TO *Puccinia polysora*

Abstract

Race-nonspecific resistance to *Puccinia polysora* was studied using RILs derived from Hi31 X Ki14. One hundred and seventeen RILs and twenty sub-lines of each parent were planted at Waimanalo, Hawaii; one hundred and seven RILs were planted at Mindanao, The Philippines to evaluate *P. polysora* resistance under natural infection. The RILs were scored in two replications at both locations using a 1 to 9 scale (1 = symptomless, 9 = all leaves killed by the fungus). At Waimanalo, the sub-lines of resistant parent Ki14 and of susceptible parent Hi31 averaged 3.29 and 7.86, and ranged from 2.0 to 5.0 and from 7.0 to 9.0, respectively. The F_1 hybrid averaged 5.5, showing intermediate resistance. The 117 RILs ranged from 2.0 to 9.0. The disease scores of the RILs ranged from 3.0 to 9.0 at Mindanao, The Philippines. One hundred and twenty-seven RFLP loci were used for QTL mapping. Five QTLs were mapped on chromosomes 2, 4, 6, 9, and 10. The QTL on chromosome 6

appeared to play a particularly important role in conditioning race-nonspecific resistance to *P. polysora*. The QTL on chromosome 10 might be, or closely link to, the previously mapped specific resistance gene *Rpp9* locus.

5.1. Introduction

Southern corn rust (*Puccinia polysora*) caused yield losses in maize in the southern part of the United States from 1972 to 1974, and was especially severe when introduced to Africa in 1949 (Rhind *et al.*, 1952). Rodriguez-Ardon *et al.* (1980) evaluated near-isogenic maize crosses and found that this fungus reduced grain yield by up to 45%. Southern corn rust was observed in Hawaii in 1975 and occasional outbreaks have caused some yield losses (Ooka, personal communication). The uredinia of *Puccinia polysora* are generally smaller, more circular in outline, and lighter red in color than those of *Puccinia sorghi*. Southern corn rust is more destructive than common corn rust, and can kill maize plants.

Storey and Howland (1957) reported two major genes conferring resistance to two races of *P. polysora*, EA. 1 and EA. 2, which appeared in Kenya. Resistance to EA. 1 was discovered in an inbred line AFRO29 (originally from

Colombia), and was governed by a monogenic dominant gene designated as *Rpp1*. A second major gene, designated as *Rpp2*, in AFRO24 of Mexican origin, was incompletely dominant and confered a lower degree of resistance to both races EA. 1 and EA. 2. Observed segregations from backcross, F_2, and F_3 populations showed that the two genes were linked with 12 crossover units separating them (Storey and Howland, 1959), but the linkage group involved was not determined.

Resistance to physiologic race PP. 9 of *P. polysora*, isolated in Indiana, is determined by a single dominant gene, designated as *Rpp9* (Ullstrup, 1965). This gene was found in maize accession PI 186208. Linkage tests from six backcross populations indicated that this gene was located on chromosome 10, about 1.6 crossover units from the *Rp1-d* locus, which governs specific resistance to *P. sorghi*. Futrell *et al*. (1975) reported that resistance to race 9 of *P. polysora* in a recovered inbred line B1138TRpp was also conditioned by a single dominant gene. The relationship of this gene to the *Rpp9* was not studied.

Scott *et al*. (1984) evaluated five F_2 populations to obtain information on the type of gene action and number of genes for resistance to *P. polysora*. These F_2 populations were from crosses between homozygous resistant selections and a common susceptible tester. They concluded that either

a one (for two selections) or two (for three selections) gene model would explain the F_2 ratios obtained. The type of gene action involved included complete, partial or no dominance. All five selections evaluated were found to have a gene for resistance at, or very closely linked with, the *Rpp9* locus.

Bailey *et al.* (1986) identified slow-rusting resistance to *P. polysora* in maize inbreds and single crosses. Zummo (1987) studied the components contributing to partial resistance to *P. polysora* in maize. He found that partial resistant genotypes had fewer pustules that were significantly smaller, less tumid, and ruptured later than those produced on susceptible genotypes. Scott and Zummo (1989) evaluated the effect of genes with slow-rusting characteristics for southern corn rust. A direct effect of fewer and smaller pustules was that smaller areas of the leaf were destroyed on resistant genotypes. The indirect effect was that fewer urediniospores were produced, and their dispersion was delayed.

Holland *et al.* (1994) studied the linkage relationships of genes conferring resistance to *P. polysora* in two maize F_2 populations using RFLP markers. RFLP probes used only covered only the regions of the genome known to contain rust resistance loci, either *Rpp* or *Rp* genes. A major gene or

genes on the short arm of chromosome 10 were identified and suggested to be alleles of *Rpp9*. Genes with smaller effects on resistance appeared to be located on chromosomes 3 and 4.

The objective of the present study was to identify the QTLs controlling general resistance to *P. polysora* with RFLP markers covering the entire maize genome.

5.2. Materials and Methods

5.2.1. Disease Nursery

One hundred seventeen RILs derived from the cross of Hi31 X Ki14 and twenty sub-lines from each parent were planted at Waimanalo, Hawaii in August, 1993 to evaluate the *P. polysora* general resistance. One hundred and seven RILs from Hi31 X Ki14 were also planted without the parental sub-lines at Mindanao, The Philippines in 1993 by Dr. M. Logrono. A 11^2 lattice design with two replications was planted at both locations. The trials were carried out under natural infection in a year-round corn cultivation condition which permits continuous epibiotics of pathogens. Rust severity was evaluated at one to two weeks after mid-silking on a rating scale of 1 to 9, with 1 indicating no pustule development and 9 indicating all leaves killed by

the fungus. An increase in the rating from 1 to 9 indicated a progressively greater proportion of the leaf surface covered by rust pustules.

5.3. Results and Discussion

5.3.1. Disease Resistance Test

RILs derived from Hi31 X Ki14 were evaluated with their parents for the race-nonspecific resistance to southern corn rust at Waimanalo, Hawaii, and Mindanao, Philippines. At Waimanalo, the sub-lines of resistant parent Ki14 and susceptible parent Hi31 averaged 3.29 and 7.86, and ranged from 2.0 to 5.0 and from 7.0 to 9.0, respectively. The F_1 hybrid averaged 5.5, showing intermediate resistance. The 117 RILs ranged from 2.0 to 9.0 (Figure 5.1). The RILs could be classified roughly into two groups as resistant lines (with disease scores lower than or equal to 5.5) and susceptible lines (with disease scores higher than 5.5). The distribution of RILs fitted a one gene model tested by the normal frequency curve method (Moon, 1995).

The disease scores of the RILs ranged from 3.0 to 9.0 at Mindanao, Philippines (Figure 5.2). The data were interpreted by Moon (1995) to be distributed in a bimodal

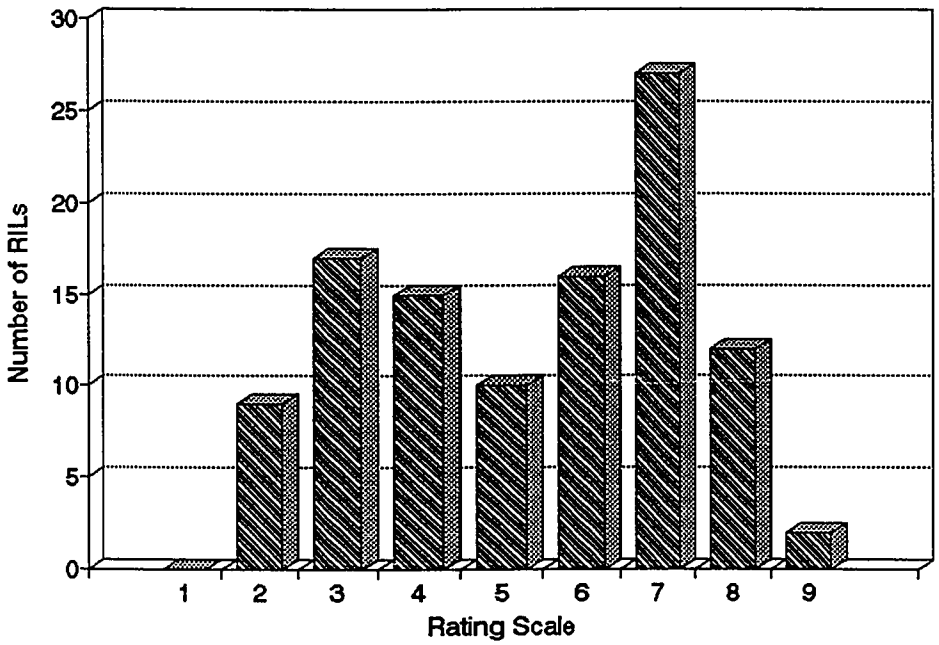


Figure 5.1. Distribution of 117 RILs of maize (Hi31 X Ki14) for reactions to *puccinia polysora* at Waimanalo, Hawaii.

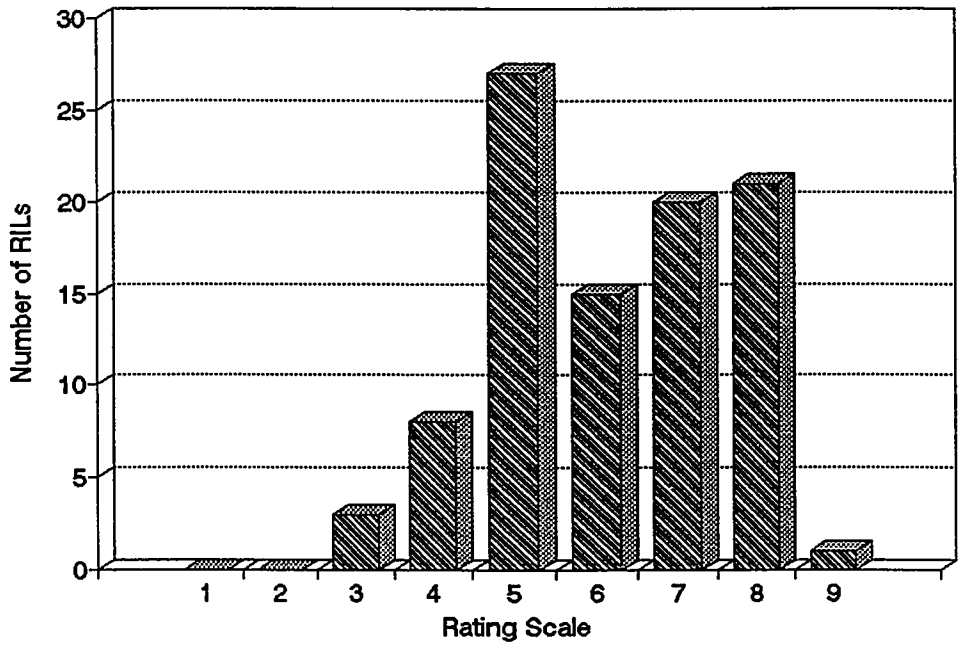


Figure 5.2. Distribution of 107 RILs of maize (Hi31 X Ki14) for reactions to *puccinia polysora* at Mindanao, Philippines.

curve that had both resistant and susceptible groups, but with more intermediate phenotypes. Least squares estimates based on predicted normal distributions indicated that the observed data fit a single gene model (Moon, 1995).

5.3.2. Mapping QTLs Controlling Race-Nonspecific Resistance to Southern Corn Rust

One hundred twenty-seven RFLP loci were used to map the QTLs for general resistance to southern corn rust. The results from single factor analysis of variance showed seven chromosomal regions correlated significantly with resistance (Table 5.1). Five chromosomal regions showed significant association with *P. polysora* at the 1% level based on data collected at Waimanalo. These five regions were on chromosomes 1L, 4L, 5S, 6L, and 10S. Data obtained at Mindanao, did not match the ones at Waimanalo for most RFLP markers associated with resistance, and only one marker on 7S reached the one percent significant level.

The combined data from both locations were also analyzed using single factor analysis of variance (Table 5.1). Average disease scores were used when disease readings of a RIL were lower or higher than the average score at both locations. If disease readings of a RIL at

Table 5.1. Loci associated significantly with P. polysora resistance from single factor analysis of variance

Marker	Chrom.	Position*1	Waimanalo R^2	UPLB R^2	Combined R^2
umc67	1	114	0.1025*		
bnl5.59	1	135	0.0714*		0.0964**
npi238	1	247	0.1502**		
npi287	2	60	0.0712**		0.0932*
umc55	2	129			0.0628*
umc5	2	145	0.0827*	0.0944*	0.1382**
umc36	2	197		0.0958*	
umc32	3	0			0.0655*
umc50	3	50		0.1078*	
umc39	3	127	0.0720*	0.0565*	0.0998*
umc96	3	173	0.0484*		
php20725	4	0	0.0995**		
umc200	4	31	0.0691*		
umc19	4	123	0.1149**		
bnl6.25	5	3		0.1103*	
umc72	5	40	0.0522*		
umc43	5	60	0.1603**		
npi393	6	30	0.0513*		0.1066**
umc21	6	106	0.1448***		
umc170	6	117	0.2033***		0.1702***
umc173	6	124	0.1175**		0.1253**
umc132	6	156	0.0582*		
umc62	6	166	0.1461**		
php20581	7	26		0.1499***	0.0850*
bnl14.07	7	105		0.0450*	0.0977*
bnl8.39	7	120		0.0993*	
umc113	9	12	0.0643*		
umc81	9	78		0.0443*	
umc95	9	101			0.0686*
bnl5.09	9	145	0.0447*		
npi291	9	155	0.1025*		
csu50	9	184	0.0967*		
csu103	10	35			0.0604*
php06005	10	63	0.1413***		
umc44	10	122		0.0513*	

*1 the map position was based on UMC 1993 RFLP map

*2 * P = 0.05, ** P = 0.01, *** P = 0.001

two locations were in conflict, e.g., one was lower but the other was higher, this RIL was treated as a missing datum. A region on chromosome 6 showed the highest R^2 value, and two important regions were also located on chromosomes 2 and 3. The inconsistency of QTLs detected in different environments has been reported in studies of gray leaf spot resistance (Bubeck *et al.*, 1993) and agronomic traits in maize (Beavis *et al.*, 1991; Stuber *et al.*, 1992; Schon *et al.*, 1993), and can obviously reflect racial differences in a pathogen like *P. polysora* or environmental effects.

The results from MAPMAKER/QTL at Waimanalo are listed in Table 5.2. Five QTLs for resistance to *P. polysora* were detected. The QTL on chromosome 6 was the most noticeable for this disease resistance, since five tightly linked markers were detected by the single factor analysis of variance method. The QTL on chromosome 10 might be closely linked to the *Rpp9* locus on this region (Ullstrup, 1965; Holland *et al.*, 1994). The QTL on chromosome 2 was confirmed only by a linked marker. The QTLs on chromosomes 4 and 9 were also detected for resistance to three other diseases -- maize mosaic virus, Stewart's bacterial wilt, and Northern corn leaf blight. It was unknown if these QTLs were directly responsible for disease resistance or were plant vigor loci

Table 5.2. Results from MAPMAKER/QTL analysis showing marker intervals detecting significant QTLs, peak LOD score, and percent variation for *P. polysora* resistance

Interval	Chromosome	LOD score[a]	% Variation[b]
umc198-umc122	2	7.20	77.0
umc133-umc15	4	6.65	73.5
umc132-umc62	6	7.70	74.0
npi291-csu50	9	8.03	77.2
npi105-php06005	10	6.08	75.9

[a] maximum LOD score between each interval

[b] phenotypic variation explained by respective locus

indirectly influencing the host plant response. No QTL was detected from the data collected at Mindanao, The Philippines.

Twelve physiologic races of *P. polysora* are known in corn (Smith and White, 1988), and the racial diversity is not known for Hawaii or Mindanao. Eleven specific resistance genes, designated *Rpp1* to *Rpp11,* have been identified (Ullstrup, 1977). Among these 11 genes, only one gene, *Rpp9,* was located on the short arm of chromosome 10 (Ullstrup, 1965). The five QTLs detected in this RIL population were located on 5 different chromosomes. The QTL on chromosome 10 was possibly allelic to *Rpp9* as discussed by Scott *et al.* (1984). It cannot be concluded if the other four regions contained any specific resistance genes. Chromosomal regions on 4S and 10S were also reported by Holland *et al.* (1994). To confirm these QTLs for race nonspecific resistance, a larger population size is favorable.

CHAPTER 6

LINKAGE OF RFLP MARKERS TO QTLS CONFERRING RACE-NONSPECIFIC RESISTANCE TO *Exserohilum turcicum*

Abstract

RFLP markers were used to map QTLs conferring race-nonspecific resistance to *Exserohilum turcicum*. One hundred and ten RILs derived from Hi31 X Ki14 and ten sub-lines of each parent were evaluated for race non-specific resistance at Mealani, Hawaii in 1993. A clear 1:1 segregation for general resistance characterized RILs in this nursery. Ninety-five RILs from Hi31 X Ki14 were planted in two separate trials to evaluate the response to race 0 and race 1 of *E. turcicum*, respectively, at Urbana, Illinois in 1994. In these two trials, plants were inoculated about one month after planting with a conidial suspension of each of race 0 and race 1. The QTL located on chromosomal region 3S was important in conferring the race-nonspecific resistance to *E. turcicum*. The chromosomal regions on three known loci, *Ht1, Ht2,* and *HtN* with qualitative effects, did not show significant association with the general resistance.

6.1. Introduction

Northern corn leaf blight (NCLB), caused by *Exserohilum turcicum*, is an important leaf disease in the U.S. northern corn belt and other temperate regions of the world. The disease is favored by moderate temperature and high humidity. The occurrence of the disease depends on environmental conditions and the level of the disease resistance of the plants. Yield losses up to 30% have been reported (Perkins and Hooker, 1981).

Control of NCLB is achieved mainly by use of resistant germplasm (Ullstrup, 1977). Four race-specific resistance genes were known, and designated *Ht1, Ht2, Ht3,* and *HtN* (Hooker, 1963, 1975, 1977; Gevers, 1975). *Ht1, Ht2,* and *Ht3* confer chlorotic-lesion resistance and result in the reduction of the amount of necrotic tissue, fungal sporulation, and inoculum for secondary infections (Hooker and Kim, 1973; Ullstrup, 1977). *HtN* is not a chlorotic-lesion type, but a mature-plant resistance that results in lesion-free plants after flowering (Gevers, 1975). The *Ht1* gene was widely used in commercial maize hybrids (Simone, 1978), whereas *Ht2, Ht3,* and *HtN* have received only limited experimental use (Smith and Kinsey, 1980). The *Ht1* gene was mapped on chromosome 2 (Hooker, 1977; Bentolila *et al.*,

1991). *Ht2* and *HtN* were closely linked on chromosome 8 but not allelic (Zaitlin *et al.*, 1992; Simcox and Bennetzen, 1994). The linkage relationship of *Ht3* is still not identified.

Race-nonspecific resistance is expressed as a reduction of lesion number and size (Hughes and Hooker, 1971; Hooker and Kim, 1973; Jenkins and Robert, 1961). This type of resistance is effective in reducing losses in corn to NCLB since it is resistant to all presently known races (Smith and White, 1988). Resistance of NCLB appeared to be correlated with resistance of Stewart's bacterial wilt and Goss's wilt (Pataky, 1985). Brewbaker *et al.* (1989) evaluated 120 tropical maize inbreds for general resistance to 19 diseases, insects, and other pests. They concluded that general resistance to NCLB is widely distributed among tropical inbreds, and notably in those derived from the Thai composite Suwan1.

Initial attempts to map race-nonspecific resistance to NCLB in maize involved a series of translocations. Regions on the long arm of chromosome 3 (3L) and 5 (5L) and the short arm of 7 (7S) appeared significantly associated with resistance (Jenkins *et al.*, 1957; Jenkins and Robert, 1961). Brewster *et al.* (1992) reported that chromosome 3, 4S and the 6L were associated with reducing disease severity. The

latter two regions were also associated with a reduction in lesion number and incubation period. The results from generation mean analysis indicated that much of this resistance was highly heritable, involving three to six genes with predominantly additive gene action (Hughes and Hooker, 1971).

RFLP markers were used to identify the location of QTLs conferring resistance to *E. turcicum* in a $F_{2:3}$ maize population by Freymark *et al.* (1993, 1994). Chromosomal regions 1S, 3L, 5S, 7L, and 8L were associated with reducing disease severity; 1S, 3L, and 5S were associated with reduction of lesion number; 5L and 7L were associated with reducing lesion size. The contribution of loci in the vicinity of *Ht1* and *Ht2* was small relative to the loci listed above.

The objective of the present study was to use RILs and RFLP markers to obtain more precise estimates of QTLs conferring general resistance to *E. turcicum*.

6.2. Materials and Methods

6.2.1 Disease Nursery

One hundred and ten RILs derived from Hi31 X Ki14 and 10 sub-lines of each parent were evaluated by Dr. H. G. Moon

for race non-specific resistance to NCLB at Mealani, Hawaii in December 1993. The testing materials was under natural infection. A maize hybrid susceptible to NCLB, Hawaiian supersweet #9, had been planted twice as spreader rows earlier, and the testing materials were surrounded by susceptible hybrids to increase the incidence of infection.

Ninety-five RILs from Hi31 X Ki14 were also planted by Dr. J. Pataky in two separate trials to evaluate the response to race 0 and race 1 of NCLB, respectively, at Urbana, Illinois in 1994 (Moon, 1995). In these two trials, plants were inoculated about one month after planting with the conidial suspension of each for race 0 and race 1. All three trials were conducted in a randomized complete block design with two replications. The disease severity was scored at one to three weeks after silking, using a 1-9 visual rating scale as described below (Moon, 1995):

1 = No visible lesions or slight infection; a few scattered lesions on lower leaves.

3 = Light infection; moderate number of lesions on lower leaves.

5 = Moderate infection; abundant lesions on lower leaves and few on middle leaves.

7 = Heavy infection; lesions abundant on lower and middle leaves extending to upper leaves; lesions on leaf sheath and/or ear husks.

9 = Heavy infection; lesions abundant on all leaves; plant may be prematurely killed; ear infection.

6.3. Results and Discussion

6.3.1 Disease Resistance Test

At Mealani, the sub-lines of resistant parent Ki14 and susceptible parent Hi31 averaged 2.62 and 7.30, respectively, and ranged from 1.0 to 5.0 and from 6.0 to 9.0. The F_1 hybrid was rated 3.1, showing resistance to *E. turcicum*. The 110 RILs ranged from 1.0 to 8.0, and could easily be classified into two groups. The RILs with a score of less than or equal to 4.5 were grouped as resistant; the susceptible RILs were those with a score higher than 4.5 (Figure 6.1). The distribution curve corresponded closely with the pattern of 1:1 segregation. A single gene model for resistance to NCLB was proposed (Moon, 1995).

Ninety-five RILs were tested separately against *E. turcicum* race 0 and race 1 at Urbana, Illinois in the summer of 1994. Each plant was inoculated one month after planting

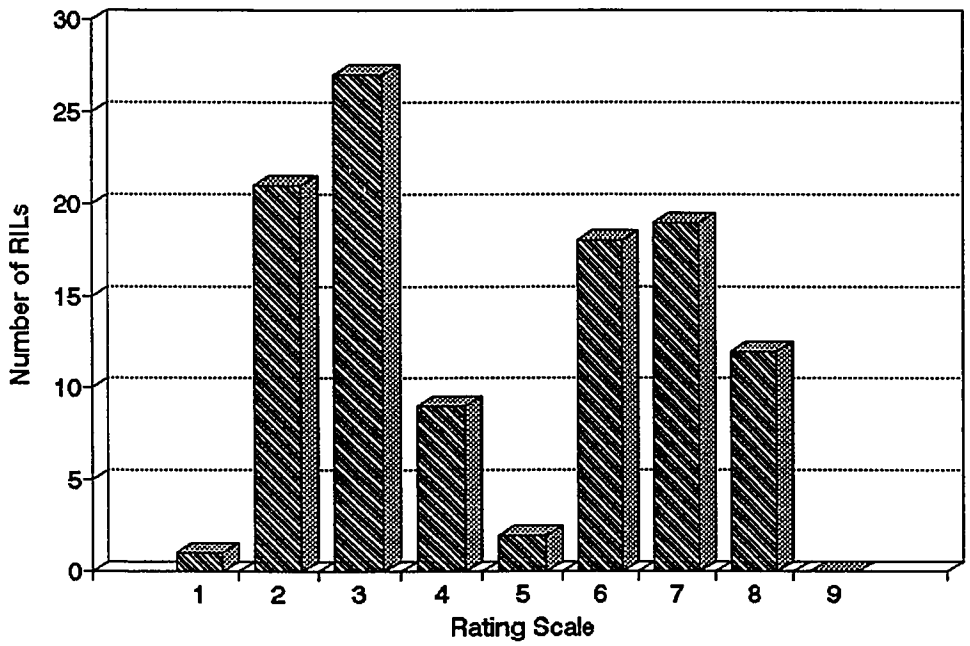


Figure 6.1. Distribution of 110 RILs of maize (Hi31 X Ki14) for reactions to *Exserohilum turcicum* at Mealani, Hawaii.

with a conidial suspension of each for race 0 and race 1. Infection was very light in the race 0 trial, the sub-lines of Ki14 and Hi31 averaged only 2.06 and 4.68, respectively, and ranged from 1.5 to 3.0 and from 3.5 to 6.5. The F_1 was rated 1.57, indicating resistance. The averages of the RILs ranged from 1.0 to 5.0 and showed no clear susceptible group (Figure 6.2).

In the race 1 trial, the sub-lines of Ki14 and Hi31 averaged 2.05 and 5.42, and ranged from 1.0 to 3.0 and from 3.5 to 6.5 (Figure 6.3). The F1 was scored 2.11, showing resistance. The average score of the RILs ranged from 1.0 to 5.0. No segregation pattern was evident. The poor blight infection score at Urbana was reportedly due to the dry field conditions in the summer of 1994.

6.3.2. Mapping QTLs Conferring Race-Nonspecific Resistance to *E. turcicum*

One hundred twenty-seven RFLP loci throughout ten chromosomes were used to map the QTLs controlling resistance to NCLB. The SAS/GLM procedure was employed to determine the associations between RFLP markers and the NCLB general resistance at the 0.05, 0.01 and 0.001 level in the three

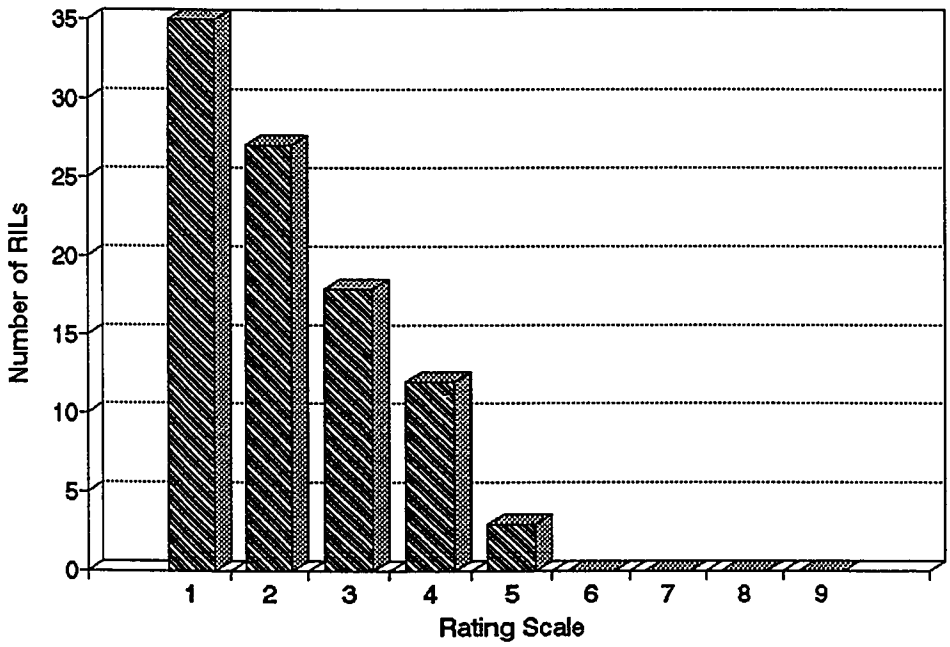


Figure 6.2. Distribution of 95 RILs of maize (Hi31 X Ki14) for reactions to *Exserohilum turcicum* race 0 at Urbana, Illinois.

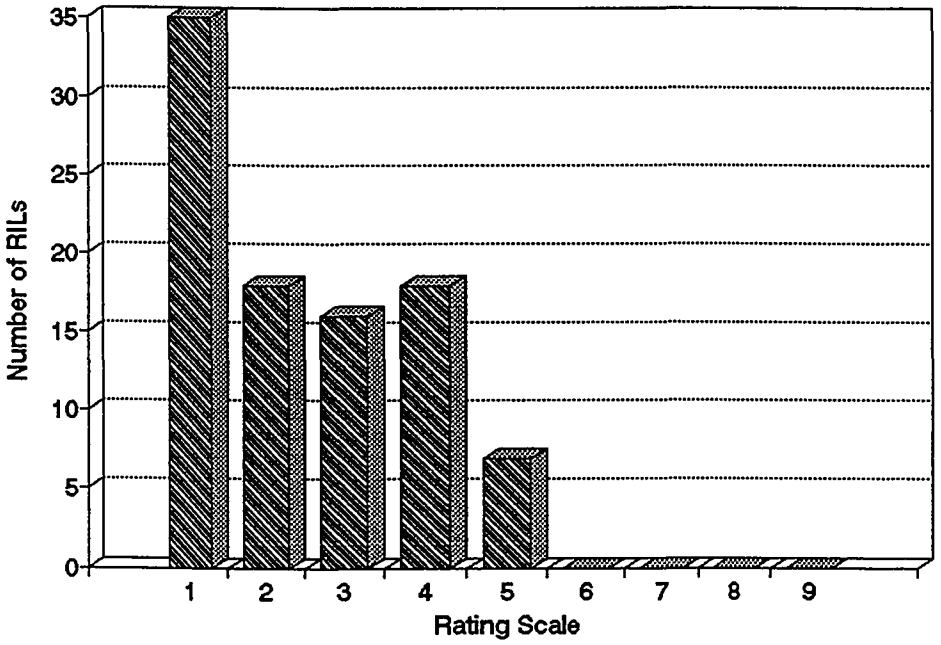


Figure 6.3. Distribution of 95 RILs of maize (Hi31 X Ki14) for reactions to *Exserohilum turcicum* race 1 at Urbana, Illinois.

trials. The program of MAPMAKER/QTL was also performed, but no QTL was identified at the LOD threshold 6.0.

The locations and effects of QTLs associated with general NCLB resistance are summarized in Table 6.1. At the Mealani trial, three regions on 2S, 3S, and 3L were associated with NCLB resistance at the one percent level. The most noticeable QTL was located on chromosome 3 with R^2 = 0.14, where another three maize disease resistance genes *rp3, wsm2,* and *mv,* are also located (see Chapter 3).

In the trials at Illinois, the results from race 0 and race 1 were similar. Chromosomal regions 1S, 4S, 4L, 7S, and 8S were important for resistance to both race 0 and race 1, whereas chromosomal region 3S for race 0 and 2S for race 1 differed from each other. Because of the poor infection, the reliability of these results is questionable.

The combined data from the three trials were also analyzed using single factor analysis of variance. The average disease score was used as combined data when disease readings of an RIL at the three trials were lower or higher than each average score. If the disease readings of an RIL at three trials conflicted, e.g. one was lower than average but another was higher than average, this RIL was treated as missing data. The results are also listed in Table 6.1. A region on the short arm of chromosome 3 exhibited the

Table 6.1. R ^ 2 values for loci significantly associated with E. turcicum general resistance

Marker	Chrom.	Position*1	Mealani	Illinois Race 0	Illinois Race 1	Illinois Combined
umc164	1	4	0.0456*			
umc157	1	20			0.0789*	
npi262	1	84		0.0569*	0.0629*	
umc167	1	107		0.1524**	0.2163**	0.1343*
bnl8.29	1	264				0.1480**
umc84	1	282	0.0748*	0.0886*	0.0965*	0.1545**
bnl6.32	1	305		0.0952*		0.1703***
umc53	2	13	0.1284**	0.1092*		0.1288*
npi287	2	60			0.1374**	0.0657*
umc131	2	94	0.0836*	0.0935*	0.1043**	0.1171**
umc198	2	155		0.0459*		
csu16	3	40		0.0787*		0.1216**
php20024	3	45				0.1389**
umc50	3	60	0.1361**	0.1394**	0.1070*	0.2529***
bnl5.37	3	92		0.0877*	0.0571*	0.0864*
umc2	3	180	0.1103**			
umc200	4	31		0.0728*		
umc193	4	69	0.0796*			
umc156	4	90		0.1694***	0.1108**	0.1137**
umc19	4	123		0.0770*		0.0692*
php20608	4	183		0.1124**	0.0981**	0.1144**
bnl8.23	4	200		0.0498*		
umc43	5	60		0.1267**	0.1003*	
csu173	5	117		0.1397*	0.1309*	0.1357**
umc104	5	214	0.0689*			
umc173	6	124	0.0615*			
umc132	6	156			0.0543*	
umc62	6	166		0.0936*	0.1144*	
umc134	6	182		0.0656*		
bnl14.07	7	105	0.0680*		0.0785*	
bnl8.39	7	120		0.2032***	0.1679***	
bnl16.06	7	143		0.1394**	0.0954*	
umc35	7	165		0.1104*		
npi114	8	1	0.0935*	0.0553*	0.0596*	0.1551**
umc103	8	31	0.0605*	0.2047***	0.2086***	0.1579**
bnl7.50	9	86			0.0643*	0.0930*
umc190	9	96	0.0963*	0.1239**	0.1206**	0.1755**
bnl7.50	9	101	0.1102*			
bnl5.09	9	145	0.1149*	0.1121**	0.0797*	0.0848*
npi291	9	155		0.2982***	0.1972**	0.2529***
csu50	9	184		0.1346**	0.1245**	
php06005	10	63		0.0993**	0.1023**	0.1068*

*1 the map position was based on UMC 1993 RFLP map
*2 * P = 0.05, ** P = 0.01, *** P = 0.001

highest R^2 value and significance level.

The chromosomal regions on three known loci, *Ht1, Ht2,* and *Htn,* with qualitative effects, did not show any association with the general resistance to NCLB. Freymark *et al.* (1993) also found that these regions exhibited relatively small effects for resistance. These results might be an exception to Robertson's hypothesis (Robertson, 1985). The chromosomal region 3S was important in conferring the race-nonspecific resistance to NCLB from the results of this study. Three regions (1S, 3S, and 7L) have been reported by Freymark et al. (1993; 1994) as affecting the disease severity in $F_{2:3}$ lines derived from B52/Mo17. In the same papers, they also reported chromosome regions 3L and 8L associated with disease severity, which did not appear in the RILs derived from tropical inbreds Hi31 and Ki14. The difference of QTLs for NCLB general resistance may be explained by the diversity between temperate and tropical germplasms.

Appendix 1. Response of RILs derived from Hi31/Ki14 for resistance to maize mosaic virus

NURSERY LOCA.: Waimanalo, Hawaii

DISEASE: MMV
SET G: Hi31/Ki14

Planting Date: April 12, 1994
Evalation date: June 29, 1994

RCB Design

MMV (1 Res. - 9 Susc.)

Plot No.	Inbred		1	2	3	4	5	6	7	8	9	10	AVG
101	G	1	6	7	5	4							5.5
314	G	1	3	1	1	4	5	1	1				2.29
315	G	2	3	4	5	6	6	6	4	3	7	5	4.9
102	G	2	3	5	3	2	8	4	3	5	5	4	4.2
316	G	3	7	3	8	8	4	8	7	6	7	7	6.5
103	G	3	1	1	1	8	2	8	6	6	7	5	4.5
317	G	4	2	2	4	2	7	7	6	6	7	7	5
104	G	4	2	1	1	1	1	4	8	7	2	3	3
318	G	9	1	1	1	6	6	8	1	1	1	6	3.2
105	G	9	7	3	4	1	1	1	1	4	3	4	2.9
106	G	13	1	1	1	1	1	1					1
359	G	13	5	3	3	1	1	1	1	1	1	1	1.8
360	G	16	7	7	7	7	7	7	7	7	7	7	7
107	G	16	2	2	3	4	6	8					4.17
108	G	17	1	1	1	1	1	1	1	1	1	1	1
361	G	17	1	1	1	1	1	1	1	1	1	1	1
109	G	21	4	1	1	1	3	1	2	1	1		1.67
362	G	21	6	6	6	6	7	7	7	7	7	7	6.6
363	G	22	6	5	1	1	1	1	1	1	1	1	1.9
110	G	22	1	1	1	1	1	1	1	1	1	1	1
111	G	23	1	1	1	1	1	1	1	1	1	1	1
364	G	23	5	5	1								3.67
365	G	25	1	1	1	1	1	1	1	1	1	1	1
112	G	25	1	1	1	1	1	1	1	1	1	1	1
333	G	26	6	6	7	6	6	7	6	8	1	2	5.5
113	G	26	6	6	1	7	7	8	7	2	8	2	5.4
336	G	28	6	6	1	6	5	6	6	7	7	8	5.8
114	G	28	7	8	6	2	6	6	5	7	8	8	6.3
115	G	29	1	1	1	3	1	1	1	6	1	1	1.7
389	G	29	1	1	1	1	1	1	1	1	1	1	1
390	G	31	1	1	1	1	6	7	1	5			2.88
116	G	31	1	1	1	1	1	1	1	1	1	1	1

Appendix 1. cont.

			MMV (1 Res. - 9 Susc.)										
Plot No.	Inbred		1	2	3	4	5	6	7	8	9	10	AVG
117	G	32	1	1	1	1	1	1	1	1	1	1	1
391	G	32	1	1	1	1	1	1	1	1	1	1	1
301	G	33	8	6	7	6	6	8	8	8	7	7	7.1
118	G	33	7	7	7	8	3	7	7	7	7	8	6.8
302	G	34	1	7	2	1	1	7	2	1	1	6	2.9
119	G	34	1	1	1	1	1	4	1	1	3	1	1.5
303	G	35	3	1	1	4	3	3	2	2	1	2	2.2
120	G	35	4	7	3	7	1	1	8	1	2	3	3.7
121	G	36	8	7	6	7	1	1	6	6	1	6	4.9
351	G	36	7	7	7	7	7	7	7	7	7	7	7
122	G	37	2	1	2	2	1	2	1	8	8	5	3.2
352	G	37	4	1	4	3	5	1	3	2	5	4	3.2
377	G	38	1	1	1	1	1	1	1	1	1	1	1
123	G	38	1	1	1	1	1	1	1	1	1	1	1
124	G	39	1	1	1	1	1	1	1	1	1	1	1
348	G	39	7	8	8	7	1	1	7	1	7	6	5.3
349	G	41	1	1	1	1	1	1	1	1	1	1	1
125	G	41	1	1	1	1	1	1	1	1	1	1	1
350	G	44	4	4	1	1	1	1	1	1	1	1	1.6
126	G	44	1	1	1	1	1	1	1	1	1	1	1
127	G	45	1	3	1	2	1	2	1	8	8	5	3.2
354	G	45	1	1	1	1	1	1	1	1	1	1	1
355	G	46	1	1	1	1	1	1	1	1	1	1	1
128	G	46	1	1	1	1	1	1	1	1	1	1	1
356	G	47	1	1	1	1	1	1	1	1	1	1	1
129	G	47	1	1	1	1	1	1	1	1	1	1	1
130	G	49	6	6	5	4	9	8	6	5	8	8	6.5
328	G	49	6	7	6	8	7	7	8	2	1	2	5.4
131	G	52	6	9	5	5	7	5	5	6	6	6	6
329	G	52	8	7	7	8	8	8	5	8	7	8	7.4
330	G	53	7	7	7	7	7	7	8	6	7	7	7
132	G	53	6	6	7	7	6	5	5	6	7	7	6.2
331	G	54	8	6	4	5	7	8	8	7	6	8	6.7
133	G	54	8	8	8	7	8	7	7	7	8	7	7.5
134	G	55	1	1	1	1	1	1	1	1	1	1	1
332	G	55	1	1	1	1	1	1	1	1	1	1	1
337	G	56	1	1	1	1	1	1	1	1	1	1	1
135	G	56	3	1	3	1	1	1	1	1	1	1	1.4
338	G	57	1	1	1	1	1	1	1	1	1	1	1
136	G	57	1	1	1	1	1	1	1	1	1	1	1
339	G	58	7	7	7	7	7	7	7	7	7	7	7
137	G	58	2	5	6	5	9						5.4

Appendix 1. cont.

			MMV (1 Res. - 9 Susc.)										
Plot No.	Inbred		1	2	3	4	5	6	7	8	9	10	AVG
138	G	59	7	1	6	5	7	5	1	7	6	5	5
340	G	59	6	6	6	6	6	6	6	6	6	6	6
347	G	60	1	7	1	1	6	6	5	2	4	2	3.5
139	G	60	6	4	6	8	7	7	7	7	6	5	6.3
346	G	63	1	2	3	1	1	3	4	2	4	1	2.2
140	G	63	1	2	1	1	1	1	1	1	1	1	1.1
141	G	64	1	6	7	5	6	4	1	2	1	6	3.9
319	G	64	1	1	1	6	6	8	1	1	1	6	3.2
142	G	65	1	1	1	1	1	1	1	1	1	1	1
342	G	65	1	1	1	1	1	1	1	1	1	1	1
143	G	66	7	8	7	8	7	2	7	7	7	7	6.7
343	G	66	1	1	3	2	6	6	5	8	7	8	4.7
144	G	67	1	1	1	1	1	1	1	1	1	1	1
344	G	67	1	1	1	1	1	1	1	1	1	1	1
345	G	68	1	1	1	1	1	1	1	1	1	1	1
145	G	68	2	2	1	1	1	1	1	1	1	1	1.2
146	G	69	5	6	8	1	5	6					5.17
323	G	69	6	6	8	4	7	6	6				6.14
147	G	70	7	8	4	5	7	8	6	7	8	8	6.8
324	G	70	1	1	1	4	1	5	1	1	5	6	2.6
148	G	72	7	8	6	8							7.25
325	G	72	1	6	1	6	6						4
326	G	73	7	1	1	8	6	1	5	4	6	7	4.6
149	G	73	7	7	7	7	8	8	8	8	7	8	7.5
150	G	74	7	6	8	7	7	7	6	6	7	7	6.8
320	G	74	1	3	7	7	6	6	1	1	6	7	4.5
321	G	75	1	1	1	1	1	1	1	1	1	1	1
151	G	75	3	1	1	2	2	1	3	1	1	1	1.6
322	G	76	1	1	1	1	1	1	1	1	1	1	1
152	G	76	1	1	1	1	1	1	1	1	1	1	1
153	G	79	6	4	5	2	6	6	6	6	7	8	5.6
341	G	79	5	5	4	7	6	6	5	5	6	1	5
327	G	80	2	1	1	3	6	1	2	1	1	1	1.9
154	G	80	7	8	7	7	6	5	6	7	5	4	6.2
366	G	82	6	6	1	1	1	1	1	1	1	1	2
155	G	82	9	8	7	6	8	7	6	6	4	6	6.7
156	G	83	1	1	1	1	1	1	1	1	1	1	1
367	G	83	1	1	1	1	1	1	1	1	1	1	1
357	G	84	1	1	1	1	1	1	1	1	1	1	1
157	G	84	1	1	1	1	1	1	1	1	1	1	1
158	G	85	7	5	6	6	7	6	7	7	7	7	6.5
358	G	85	5	6	6	7	7	7	7	7	7	7	6.6

Appendix 1. cont.

			MMV (1 Res. - 9 Susc.)										
Plot No.	Inbred		1	2	3	4	5	6	7	8	9	10	AVG
334	G	86	1	1	1	1	1	1	1	1	1	1	1
159	G	86	5	5	1	1							3
160	G	87	1	1	1	1	1	1	1	1	1	1	1
335	G	87	1	1	1	1	1	1	1	1	1	1	1
161	G	89	1	1	1	1	1	1	1	1	1	1	1
307	G	89	1	1	1	1	1	1	1	1	1	1	1
310	G	90	1	1	1	1	1	1	1	1	1	1	1
162	G	90	1	1	1	1	1	1	1	1	1	1	1
163	G	91	2	1	1	1	1	1	1	1	1	1	1.1
308	G	91	1	2	2	4	1	1	1	1	1	1	1.5
311	G	92	2	3	1	1	1	1	1	1	1	1	1.3
164	G	92	1	1	1	1	1	1	3	3	5	5	2.2
304	G	94	2	2	7	1	2	3	7	2	2	4	3.2
165	G	94	1	5	4	1	3	2	6	6	6	1	3.5
166	G	96	6	3	3	7	7	3	7	7	5	1	4.9
305	G	96	2	1	1	1	1	1	1	1	1	1	1.1
306	G	98	5	1	1	1	6	2	7	4	1	3	3.1
167	G	98	7	9	8	7	8	8	8	8	7	7	7.7
309	G	100	7	2	8	8	6	4	3	7	7	7	5.9
168	G	100	6	6	8	8	6	5	8	4	7		6.44
312	G	101	1	1	1	1	1	1	1	1	1	1	1
169	G	101	4	1	1	1	1	1	1	1	1	1	1.3
170	G	103	4	4	6	7	8	3	3	4	7	6	5.2
313	G	103	5	5	1	1	7	6	7	6	7	7	5.2
368	G	104	7	1	7	6	5	1	1	5	4	1	3.8
171	G	104	7	7	7	8	7	7	7	7	5	7	6.9
369	G	105	8	3	1	1	1	1	1	1	1	1	1.9
172	G	105	2	8	8	2	7	7	3	6	5	6	5.4
370	G	106	7	1									4
173	G	106	7	7	7	7	7	7	7	7	7	7	7
174	G	107	7	3	3	3	4	3	2	1	2		3.11
371	G	107	1	1									1
372	G	108	1										1
175	G	108	7	6	7	1	1	3	4	5	1	3	3.8
176	G	111	7	7	6	6	7	7	5	8	8	7	6.8
373	G	111	5	6	6	4	4	1	1	1	6	7	4.1
374	G	112	1	5	1	1	1	2	1	5	5	4	2.6
177	G	112	7	7	7	7	7	7	7	7	7	7	7
375	G	114	1	1	1	1	1	1	1	1	1	1	1
178	G	114	1	1	1	1	1	1	1	1	1	1	1
376	G	115	1	1	1	1	1	1	1	1	1	1	1
179	G	115	1	1	1	1	1	1	1	1	1	1	1

Appendix 1. cont.

			MMV (1 Res. - 9 Susc.)										
Plot No.	Inbred		1	2	3	4	5	6	7	8	9	10	AVG
385	G	116	1	1	1	1	1	1	1	1	1	1	1
180	G	116	1	1	1	1	1	1	1	1	1	1	1
386	G	117	1	1	1	1	1	1	1	1	1	1	1
181	G	117	1	1	1	1	1	1	1	1	1	1	1
182	G	118	7	6	4	8	7	1	8	8	6	8	6.3
387	G	118	5	1	2	2	1	6	5	1	1	1	2.5
388	G	119	5	1	6	1	7	7	7	7	7	7	5.5
183	G	119	7	8	8	8	1	3	8	7	7	8	6.5
184	G	120	1	1	1	1	1	1	1	1	1	1	1
378	G	120	1	1	1	1	1	1	1	1	1	1	1
379	G	121	5	1	1	1	1	1	1	1	1	1	1.4
185	G	121	1	1	1	1	1	1	1	1	1	1	1
186	G	122	7	6	4	2	3	2	4	8	3		4.33
380	G	122	5	1	1	5	7	8	4	5	5	5	4.6
187	G	124	1	1	1	1	1	1	1	1	1	1	1
381	G	124	1	1	1	1	1	1	1	1	1	1	1
382	G	125	6	6	5	1	1	1	4	4	3	7	3.8
188	G	125	3	2	2	7	7	7	6	7	1	7	4.9
189	G	126	8	8	1	8	1	1	1	3	1	2	3.4
383	G	126	5	1	1	1	1	5	1	1	1	1	1.8
190	G	128	8	8	1	1	1	1	1	6	6	6	3.9
384	G	128	1	1	1	1	1	1	1	1	1	1	1
353	G	129	4	1	1	1	1	5	2	1	1	1	1.8
191	G	129	6	1	1	1	1	1	1	1	1	1	1.5

Appendix 2. Response of RILs derived from Hi31/Ki14 for resistance to fungal, bacterial and viral diseases.

Pedigree	S. Rust *1 Minda	S. Rust *1 Waima	Common Rust	N. leaf blight *2 GR	N. leaf blight *2 R 0	N. leaf blight *2 R 1	SBW*3	MMV
Hi31		7.9	7.3	7.3	4.7	5.4	6.5	2.1
Ki14		3.3	4.0	2.6	2.1	2.1	1.9	7.2
F1		5.5	6.0	3.1	2.8	3.1	4.1	
G 1	6							4.8
G 2	6	2.5	3.0	3.1	2.5	3.1		5.7
G 3		4.0	6.5	2.2	2.8	1.8	1.7	6.2
G 4	5	4.4	5.0	3.1	1.9	1.8	1.6	5.1
G 5	6	7.5		7.8				
G 6	6	7.5		3.3				
G 7	5		4.0		4.0	4.8		
G 8	5	6.4	2.0	2.3	2.0	3.1		
G 9	7	7.7	7.0	3.4	4.3	3.6	6.9	4.1
G 10	5	5.6	4.5	3.4				
G 12	6	9.0	7.0	2.8	2.6	2.0	1.5	
G 13	7		8.0		5.0	5.5	4.9	2.4
G 14	7	8.1	7.5	8.1	5.0	5.1	7.5	
G 15	5	7.0	7.5	7.8				
G 16	5	7.8		2.2				6.6
G 17	4	3.4	6.5	6.9	5.3	6.0	6.9	2.0
G 19	6	7.3		3.5				
G 20		9.0		7.9				
G 21	5	6.7	6.5	6.3	4.8	5.5	7.2	5.1
G 22	7	2.8	3.5	1.9	6.5	6.5	7.7	3.3
G 23	5	4.3	6.5	3.8	2.8	2.8		3.3
G 24	7	7.7	6.5	7.1	4.1	4.3	6.7	
G 25	8	7.1	3.0	7.3	6.0	7.9	7.2	2.0
G 26	4	5.5	3.5	4.4	6.0	7.3	6.7	6.5
G 27					3.0	3.5	1.8	
G 28	4	4.3	6.5	1.7			2.7	7.1
G 29	5	3.0	3.5	2.4	5.5	5.3		2.4
G 30		4.0		6.6	4.3	5.6		
G 31	6	8.4						2.9
G 32	5	2.7	6.5	6.4	4.8	5.5		2.0
G 33	5	5.0	6.5	2.9	5.0	4.0	1.7	8.0

Appendix 2. cont.

Pedigree	S. Rust *1		Common	N. leaf blight *2			SBW*3	MMV
	Minda	Waima	rust	GR	R 0	R 1		
G 34	8	2.5	6.5	7.8	6.3	5.6	7.9	3.2
G 35	4	3.3	4.0	2.5	4.5	6.0	3.8	4.0
G 36	5	7.0	4.0	6.5	2.1	2.0	1.5	7.0
G 37	8	5.1	6.5	6.5	5.1	5.5		4.2
G 38	7	7.3	5.0		4.5	6.4	2.6	2.0
G 39		4.4	4.0	6.5	4.0	5.5	7.0	4.2
G 40	7	6.2	3.5	2.7			3.0	
G 41	5	6.5	4.0	6.6	3.3	3.3		2.0
G 43		7.7	4.0	5.7				
G 44		3.0	4.0	2.2	2.0	2.3	1.2	2.3
G 45	4	2.2	3.5	3.5	5.3	5.3	6.5	2.2
G 46	7	5.4	6.5	3.7	4.1	5.8		2.0
G 47	8	4.3	5.5	7.4	6.5	8.5	7.2	2.0
G 48	7	7.0	5.0	2.9	4.5	7.5	7.3	
G 49	6	5.0	4.0	3.7	2.1	8.5	1.8	6.9
G 50	7	7.8		2.3				
G 51	7	7.5		8.5				
G 52		2.0	7.5	6.5				7.7
G 53	8	4.3	3.0	3.9	4.4	4.6		7.6
G 54		6.5	7.0	7.5	4.9	5.1	6.0	8.0
G 55	5	3.0	4.0	6.5	6.0	7.0	6.8	2.0
G 56		2.9	4.0	6.0	5.3	6.0		2.2
G 57	4	2.0	4.5	1.4	2.5	2.3	1.5	2.0
G 58		6.0	4.0	2.1	4.0	2.8	1.3	7.1
G 59	7	7.0		3.1	3.8	3.0	1.3	6.5
G 60	8	6.0	5.0	1.6	3.3	3.4	1.9	5.9
G 62		4.3	5.0	2.6	3.8	4.4	5.3	
G 63	9	7.7	7.0	2.9	4.5	3.9	6.9	2.7
G 64	7	6.8	7.0	2.1	2.0	1.9		4.5
G 65	8	3.3		3.6	3.9	2.0	2.0	2.0
G 66	8	7.5	8.0	7.2	2.8	3.3	4.4	6.7
G 67	8	4.3		6.4	6.5	5.9	6.7	2.0
G 68	8	7.0	6.5	6.2	3.9	4.6	6.8	2.1
G 69	8	8.2	8.0	7.8				6.7
G 70		7.5	6.5	3.2	5.3	6.4	7.2	5.7
G 71	6	7.0	4.0	6.8				
G 72	8	7.7		7.5				6.6

Appendix 2. cont.

Pedigree	S. Rust *1 Minda	S. Rust *1 Waima	Common rust	N. leaf blight *2 GR	N. leaf blight *2 R 0	N. leaf blight *2 R 1	SBW*3	MMV
G 73	6	6.0			4.9	4.0	2.8	7.1
G 74	5	6.0	5.0	2.4	1.8	1.9	1.9	6.7
G 75	7	6.6	7.5	3.4	5.1	5.4	5.2	2.3
G 76		7.6	6.5	6.9	3.9	4.4		2.0
G 78	6	4.2	6.5	6.5	5.1	5.1	2.8	
G 79	7	3.2	4.0	2.7	4.8	4.3	6.3	6.3
G 80		5.6	6.5	2.7	6.0	5.6	6.1	5.1
G 82		2.3	3.0	3.7	4.5	6.4		5.3
G 83	8	5.0	4.0	2.7	8.3	6.5		2.0
G 84	5	7.0	5.5	7.3	3.4	2.5		2.0
G 85	6	2.3			3.4	2.5	3.0	7.6
G 86	8			5.6				3.3
G 87	8	8.9	7.5	6.9	4.8	6.0	6.1	2.0
G 88	8	7.0		7.4	2.9	2.6	2.0	
G 89	8	7.8	7.0	1.8	2.8	3.1		2.0
G 90	5	6.6	3.5	7.7	5.0	5.9	6.1	2.0
G 91	7	3.0	3.5	3.9	5.0	5.3	3.2	2.3
G 92	5	2.0	4.0	5.7	3.8	5.5		2.8
G 94	5			5.6	2.1	3.5	2.0	4.4
G 95	8	6.1	8.0	6.6	3.4	3.6	4.2	
G 96	7	3.0	6.0	7.8	4.4	7.0		4.0
G 97	8			4.0	2.5	2.3		
G 98		7.5	6.5	2.7	2.5	1.6	1.8	6.4
G 99	5	4.3	4.0	2.3				
G 101	7				3.8	4.5		2.2
G 102	8	6.4	4.0	7.7				
G 103	7			3.2	2.5	2.3	1.4	5.9
G 104	5	7.5	3.5	2.4	3.0	2.4	3.6	6.4
G 105	8	4.0	5.5	1.9	1.8	2.0	1.8	4.7
G 106	5	7.5	5.0	1.7	2.9	3.0	3.2	6.5
G 107	3	3.3	3.5	2.9	4.9	4.5		3.1
G 108	4	2.0	2.0	2.8	2.9	2.0	2.0	3.4
G 111	5	7.1	7.0	2.3	2.9	2.4	3.0	6.5
G 112		7.5	5.5	2.6	4.8	3.0	3.8	5.8
G 113	6	6.0		2.3				
G 114	6	3.0	4.0	3.2	5.0	6.0		2.0

Appendix 2. cont.

Pedigree	S. Rust *1 Minda	S. Rust *1 Waima	Common rust	N. leaf blight *2 GR	N. leaf blight *2 R 0	N. leaf blight *2 R 1	SBW*3	MMV
G 115	5	6.0		3.6	4.9	6.6	6.2	2.0
G 116	4	5.1	5.0	7.4	5.3	4.9	6.5	2.0
G 117	5	3.0	4.0	3.0	4.5	5.3		2.0
G 118		6.6	6.5	5.5	2.6	2.4	1.6	5.4
G 119	5	6.5	3.0	4.8	3.4	5.0	6.1	7.0
G 120	4	5.3	6.0	6.4	3.0	2.6		2.0
G 121	5	3.3	4.5	6.8	4.0	2.1	6.5	2.2
G 122		3.9	7.5	8.3	5.5	4.5	6.2	5.5
G 124		6.5	6.5					2.0
G 125	3	3.3	2.0	2.2	3.4	3.3	1.7	5.4
G 126	3	4.0	3.5	6.6	6.3	6.0		3.4
G 127	7	7.0	7.0	8.0	6.6	7.5	6.5	
G 128	5	5.0	3.5	5.7	6.9	7.0	6.9	3.5
G 129		6.5	6.0	7.4	3.6	3.0	3.8	2.7
Count	105	115	99	117	101	101	73	93
Mean	5.6	5.2	4.9	4.3	3.9	4.2	4.2	4.0
STD	2.2	2.2	2.0	2.4	1.6	2.0	2.4	2.1
CV (%)	23.8	35.3	40.6	56.2	42.3	48.3	57.1	48.2

All diseases were recorded on the rating scale of 1-9.

*1 S. rust was scored at Mindanao (Philippines) and Waimanalo.

*2 N. leaf blight was scored at Mealani (HI) for GR (general resistance) of N. leaf blight and at Urbana (IL) for R 0 (race 0) and R 1 (race 1).

*3 SBW (Stewart's bacterial wilt) was recorded at Henderson (KY) and others at Waimanalo.

Appendix 3. Data of 127 RFLP markers on 117 RILs of maize (Hi31 X Ki14).

(A = Hi31 allele, B = Ki14 allele, C = non-parental allele, H = heterozygous locus, O = outlier, - = missing data)

	Restric. Enzyme	RFLP Marker	RILs 1 G1	2 G2	3 G3	4 G4	5 G5	6 G6	7 G8	8 G9	9 G10	10 G12	11 G13	12 G14	13 G15	14 G16	15 G17	16 G19	17 G21	18 G22	19 G23	20 G24
1	HindIII	umc39	A	A	A	B	A	B	A	B	B	A	A	B	B	A	B	A	A	A	B	A
2	HindIII	bnl8.29	B	A	A	B	A	B	A	B	A	A	A	B	B	A	B	B	B	B	A	B
3	HindIII	bnl8.29b	A	A	A	B	B	A	A	H	B	A	A	B	B	A	A	B	B	A	A	A
4	EcoRI	npi285	-	A	-	A	B	A	A	A	B	B	B	B	-	B	A	B	B	O	B	A
5	HindIII	umc5	B	B	-	A	-	A	B	A	A	A	A	A	B	A	B	-	-	B	B	B
6	EcoRV	umc32	B	A	A	A	B	A	B	B	B	A	A	A	B	B	B	A	A	A	B	A
7	EcoRV	umc89	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	B	B
8	EcoRV	bnl6.32	B	A	B	A	A	A	A	B	A	A	A	B	O	A	B	B	B	B	-	B
9	SstI	umc200	A	A	B	A	B	A	A	A	A	A	B	A	A	A	A	H	B	A	A	A
10	EcoRI	umc21	B	B	B	B	A	A	B	A	B	A	B	B	A	B	A	A	B	B	A	A
11	DraI	umc107	A	A	A	B	A	B	A	B	A	A	A	A	A	A	B	B	A	B	A	B
12	EcoRV	umc167	A	A	B	A	B	B	-	A	A	B	A	A	-	B	A	B	A	A	-	-
									C												C	C
13	SstI	npi268	B	B	A	A	B	B	B	A	B	B	B	A	A	A	B	A	B	A	B	A
14	SstI	npi268b	B	B	A	B	B	A	B	B	B	A	A	A	B	B	A	A	B	B	A	A
15	EcoRV	umc11	B	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	A	B	B	B
16	EcoRI	php06005	A	H	A	A	B	B	B	B	A	B	H	B	A	B	B	B	B	A	A	B
17	DraI	umc170	B	B	B	B	B	A	B	A	A	A	B	B	A	A	A	A	B	B	A	A
18	SstI	php20855	A	A	A	B	B	A	B	B	A	A	A	A	A	B	B	B	A	B	A	B
19	HindIII	umc68	-	-	-	B	A	B	A	B	A	B	B	A	A	A	A	A	B	A	B	B
20	EcoRI	umc204	A	B	A	H	A	B	A	A	B	A	B	B	B	A	A	B	B	B	B	A
21	DraI	umc2	B	A	B	B	B	B	B	B	B	B	B	A	A	B	B	B	B	B	B	A
22	BamHI	umc147	B	B	A	A	B	A	B	A	B	A	A	B	B	A	A	B	B	A	B	B
23	BamHI	umc147b	A	A	A	B	A	B	A	-	A	B	A	B	A	A	-	B	B	-	A	B
24	EcoRI	umc16	-	A	A	B	B	B	-	-	A	A	A	A	A	B	-	A	B	-	-	-
25	EcoRI	umc16b	B	B	A	B	A	A	B	A	B	A	A	A	A	B	B	B	-	B	B	A
			C						C	C							C			C	C	C
26	EcoRI	umc193	B	B	A	A	B	B	B	A	B	B	B	B	A	A	B	A	B	A	B	A
27	EcoRI	umc193b	B	B	H	H	B	A	H	B	B	B	A	B	B	B	B	A	H	B	H	B
										C							C			C	C
28	EcoRV	bnl12.06	B	A	A	A	A	A	B	A	B	A	A	B	B	B	A	B	A	A	B	B
											C				C	C	C	C				
29	EcoRV	umc67	A	A	A	B	B	B	-	A	A	B	B	B	A	B	-	B	A	A	-	-
									C								C				C	C
30	BglII	php10017	A	A	A	A	A	B	A	A	B	A	A	B	H	A	-	A	B	A	-	-
31	BamHI	umc51	B	B	B	B	A	A	B	A	A	B	B	A	B	B	B	H	B	A	B	B
			C						C												C	C
32	HindIII	umc104	A	A	A	A	A	B	A	A	B	A	A	B	B	A	B	A	B	A	B	B
33	HindIII	umc173	B	B	B	B	B	B	B	A	A	A	B	B	H	A	A	A	B	B	A	A
34	HindIII	umc173b	B	B	B	B	A	B	B	A	B	B	B	A	A	B	B	B	B	A	B	A
35	HindIII	umc63	-	A	B	B	B	B	A	A	B	A	A	A	-	A	-	A	A	-	A	-
			C												C		C			C		C
36	SstI	umc140	A	A	A	-	A	-	A	B	A	A	-	A	A	A	B	-	A	B	A	B
						C		C										C				
37	BamHI	umc128	A	A	A	B	B	B	B	B	A	A	A	A	A	B	B	A	B	B	A	B
38	EcoRI	php20608	H	A	A	A	B	A	A	A	A	B	A	B	B	A	B	B	B	B	B	B
39	EcoRI	php20608b	H	A	A	A	B	B	B	B	A	B	B	A	B	B	A	A	A	A	B	B
40	EcoRV	umc96	B	B	B	B	B	B	B	A	B	A	A	A	A	A	B	A	A	A	B	B
41	BamHI	npi107	A	A	A	B	A	B	A	B	B	A	A	B	B	A	B	A	A	A	B	A
42	EcoRI	umc26	A	A	B	B	B	A	A	A	B	A	A	B	B	B	A	A	B	A	A	A

Appendix 3. Cont.

	Restric. Enzyme	RFLP Marker	RILs 1 G1	2 G2	3 G3	4 G4	5 G5	6 G6	7 G8	8 G9	9 G10	10 G12	11 G13	12 G14	13 G15	14 G16	15 G17	16 G19	17 G21	18 G22	19 G23	20 G24
43	EcoRI	umc26b	B	B	A	B	A	A	A	B	B	A	A	B	A	B	A	A	B	A	B	A
44	EcoRI	umc131	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A
45	BamHI	umc134	A	A	B	A	B	A	A	A	B	B	B	A	A	A	A	A	B	A	A	A
46	SstI	umc103	A	A	A	B	A	B	A	B	B	A	A	B	H	A	B	A	A	A	B	A
47	EcoRV	umc122	A	A	A	B	B	B	A	A	B	A	A	B	A	A	A	B	A	A	A	A
						C									C							
48	SstI	bnl5.71	B	A	B	A	B	B	B	B	A	B	B	A	B	B	A	A	A	B	B	A
49	BglII	umc102	B	B	A	B	B	A	A	B	B	A	A	B	A	B	A	H	B	A	A	A
50	BamHI	npi262	A	A	A	A	B	A	A	A	A	A	A	B	A	B	A	B	A	A	A	A
51	HindIII	umc53	-	-	B	B	A	A	B	A	B	A	A	A	A	A	A	A	A	-	A	A
			C	C																C		
52	DraI	bnl6.25	H	A	A	A	B	A	A	A	A	-	A	B	H	A	-	B	B	-	-	-
												C					C			C	C	C
53	HindIII	npi238	A	-	A	B	A	B	A	-	A	A	A	B	B	A	-	B	B	-	A	-
										C							C			C		C
54	EcoRI	umc55	A	B	B	A	B	A	A	B	A	A	A	A	B	A	B	A	B	B	B	B
55	HindIII	umc133	A	A	B	-	-	A	B	A	B	B	B	A	A	-	B	A	B	A	B	B
						C	C									C						
56	HindIII	umc58	A	A	A	B	B	A	B	H	A	A	A	A	H	B	B	B	A	A	A	B
57	EcoRI	umc121	B	A	A	B	A	A	B	B	B	A	A	A	B	B	B	A	A	A	A	A
58	DraI	umc27	A	B	B	A	A	A	A	A	A	B	B	B	A	A	A	B	A	A	B	B
59	HindIII	umc120	B	B	B	B	A	B	B	B	B	B	B	A	A	B	B	B	B	B	A	A
60	DraI	umc44	A	B	A	A	B	A	B	B	A	A	B	A	A	B	B	A	B	B	H	A
61	BamHI	bnl8.39	A	A	B	B	A	A	A	A	B	B	B	A	A	B	A	A	B	A	A	A
62	SstI	umc62	-	A	B	B	A	A	-	A	B	B	B	A	-	A	-	B	B	A	A	A
			C						C						C		C					
63	BamHI	bnl5.37	B	B	A	B	B	A	B	B	B	A	A	B	A	B	A	A	B	A	A	A
			C	C					C	C												
64	HindIII	umc185	B	A	A	A	A	A	B	A	B	A	A	B	B	B	A	B	A	A	B	B
65	SstI	bnl8.23	A	A	A	B	B	A	A	A	B	B	B	A	A	H	A	B	B	A	A	A
66	BamHI	bnl7.50	A	-	A	B	A	B	A	A	B	A	A	B	-	B	-	H	A	-	-	-
				C													C			C	C	C
67	SstI	php20024	-	A	A	A	A	A	A	-	B	A	A	A	A	B	A	A	A	A	A	A
			C							C												
68	HindIII	umc124	-	-	-	A	A	A	B	B	A	B	B	A	A	A	B	A	B	B	B	B
69	EcoRI	umc84	B	A	A	A	A	A	A	B	A	A	A	B	B	A	B	B	B	B	A	B
70	BamHI	npi286	B	A	A	A	A	A	B	A	B	A	A	B	B	B	A	B	A	A	B	B
71	DraI	umc82	H	B	H	A	B	A	B	B	H	A	A	A	A	B	A	A	B	B	B	A
72	DraI	umc82b	A	A	A	A	A	B	A	A	B	B	B	B	A	A	A	A	B	A	A	A
73	EcoRV	npi97	B	B	A	A	B	A	A	B	B	B	A	B	B	B	B	A	A	A	A	B
74	SstI	umc35	A	B	A	B	B	A	B	A	B	A	A	A	A	B	A	A	B	A	A	B
75	XbaI	umc36	B	B	A	B	A	B	A	B	B	B	B	A	B	B	-	H	A	B	A	A
76	XbaI	csu173	-	-	B	A	B	B	A	H	-	B	B	H	A	B	-	A	B	A	B	A
77	EcoRV	npi414	B	B	A	A	A	B	B	B	B	B	B	B	B	A	B	A	B	B	B	B
78	HindIII	umc198	A	A	A	B	B	B	A	A	B	A	B	A	A	B	A	B	A	A	A	A
79	HindIII	umc198b	-	-	B	A	B	A	A	A	B	B	B	A	B	A	-	B	A	B	A	A
			C	C													C					
80	BglII	npi400	B	B	A	B	B	B	A	A	B	A	A	B	B	A	A	B	B	A	A	A
81	BamHI	umc81	B	B	B	A	A	B	A	A	B	B	B	B	B	B	B	B	B	A	B	B
82	EcoRI	umc117	B	B	-	B	B	B	B	B	B	B	B	A	-	B	B	B	B	B	B	A
83	SstI	umc113	B	B	B	H	B	B	A	-	B	A	B	B	A	A	A	B	B	A	A	A
84	BamHI	bnl5.59	A	A	A	B	B	A	A	A	A	B	B	B	A	B	A	B	A	A	A	A
85	DraI	umc157	H	B	B	A	H	A	A	B	H	B	B	B	H	H	H	A	A	A	A	B

Appendix 3. Cont.

	Restric. Enzyme	RFLP Marker	RILs 1 G1	2 G2	3 G3	4 G4	5 G5	6 G6	7 G8	8 G9	9 G10	10 G12	11 G13	12 G14	13 G15	14 G16	15 G17	16 G19	17 G21	18 G22	19 G23	20 G24
86	HindIII	bnl5.09	A	A	A	A	A	B	A	A	B	A	A	A	A	B	A	A	B	A	A	A
				C											C					C	C	
87	BamHI	umc54	B	B	B	H	A	B	A	B	A	B	B	A	A	B	A	A	B	A	B	A
88	EcoRI	umc13	B	A	A	B	B	B	A	A	B	A	A	A	B	A	A	B	A	A	B	A
89	EcoRV	umc156	A	A	B	B	B	A	A	A	A	B	B	A	A	A	A	A	B	A	A	A
90	BamHI	umc38	B	A	B	A	B	B	B	B	B	A	A	B	B	A	B	A	B	B	A	A
91	BamHI	umc190	A	A	A	B	A	B	A	A	B	A	A	B	A	A	A	A	A	A	A	A
92	SstI	csu86	A	A	A	B	B	A	B	B	A	A	A	A	A	B	B	B	A	B	A	B
93	EcoRI	bnl1.297	A	A	A	H	B	A	A	A	H	A	A	A	A	B	A	A	B	A	A	A
94	BglII	umc95	A	B	A	B	A	B	A	A	B	A	A	B	B	B	B	H	A	B	B	B
95	DraI	php20725	A	A	A	A	A	A	A	A	A	A	A	B	A	B	A	A	B	A	A	A
96	SstI	csu16	A	A	A	B	A	A	A	A	B	A	A	A	A	B	A	A	A	A	A	A
97	SstI	umc72	A	A	A	A	B	A	-	A	A	A	A	A	A	B	-	B	A	A	A	-
									C								C					C
98	EcoRV	umc43	-	A	B	A	B	B	-	-	B	B	B	A	A	A	-	A	B	A	-	-
			C						C	C							C				C	C
99	SstI	umc50	-	-	A	-	A	A	A	-	B	A	A	A	A	B	A	A	B	A	-	A
			C	C		C				C											C	
100	HindIII	npi291	A	-	B	B	A	B	A	A	B	B	B	B	A	B	A	A	B	-	-	A
				C																C	C	
101	HindIII	npi291b	A	A	A	A	B	B	B	B	B	A	A	A	B	B	B	B	B	A	A	B
102	HindIII	csu59	B	B	A	B	A	B	A	A	B	A	B	B	B	A	B	B	B	B	B	A
103	BamHI	umc15	-	-	A	A	B	A	A	A	A	B	B	A	B	A	-	A	A	B	A	A
			C	C													C					
104	BamHI	umc110	A	A	B	A	A	A	B	A	A	A	A	A	B	A	A	A	A	A	B	A
105	HindIII	csu133	B	A	B	B	A	A	B	B	B	A	A	A	B	A	A	B	A	B	A	B
106	EcoRI	php20581	B	B	A	B	A	B	A	B	B	B	B	A	B	B	B	A	A	B	A	A
107	EcoRI	php20581b	B	A	A	B	B	B	A	A	B	A	A	B	A	A	A	A	B	A	A	A
108	EcoRI	npi105	B	B	-	B	-	A	-	B	B	A	A	B	-	B	B	-	B	A	B	-
					C		C		C						C			C				C
109	HindIII	npi287	A	B	B	B	A	A	B	A	A	A	A	A	B	A	A	B	A	B	A	A
110	HindIII	php20508	B	B	B	B	B	A	A	B	B	A	A	B	A	B	A	A	B	A	A	A
111	SstI	UMC109	A	B	A	A	A	B	B	B	B	A	B	B	A	A	A	B	B	B	B	A
112	BglII	bnl16.06	A	A	B	B	B	A	A	A	B	B	B	A	A	B	-	A	B	A	-	-
																	C				C	C
113	BglII	bnl16.06b	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B	B	B	A
114	EcoRI	umc136	B	A	A	B	B	A	B	A	A	B	B	A	B	B	B	B	A	A	B	A
115	EcoRI	bnl14.07	A	A	B	B	H	A	B	A	B	B	B	A	A	B	A	A	O	A	B	A
116	HindIII	csu50	A	A	A	A	A	A	A	A	B	A	A	A	B	A	A	A	B	A	A	A
117	SstI	php20075	A	A	A	A	B	A	A	A	B	B	B	B	A	B	A	B	B	A	A	A
118	BamHI	umc132	A	A	B	B	B	B	A	A	A	A	A	B	A	A	A	A	B	A	A	A
119	DraI	umc19	A	A	B	A	B	A	H	A	A	B	B	A	A	B	A	B	A	A	A	A
120	BamHI	npi393	-	B	A	B	A	A	B	B	A	B	B	A	A	B	B	A	A	B	B	A
121	BamHI	umc123	A	A	A	A	A	B	B	B	A	A	A	A	A	A	B	A	A	A	B	B
122	BamHI	csu30	B	B	B	B	B	A	A	B	B	A	A	B	A	B	A	A	B	A	A	A
123	EcoRI	npi239	-	-	A	B	A	A	A	A	B	A	A	A	A	A	A	A	A	-	A	A
			C	C																C		
124	HindIII	umc103	A	-	B	B	B	B	A	A	B	B	B	A	A	A	A	A	B	A	A	A
125	EcoRV	umc164	B	B	A	A	B	A	B	B	B	B	A	B	B	B	B	A	B	B	A	B
126	DraI	npi321	A	B	A	A	B	A	B	A	B	A	B	A	-	B	A	A	B	B	A	A
127	SstI	npi114	B	B	A	A	A	A	A	B	B	A	A	B	A	B	A	A	B	A	B	A

Appendix 3. Cont.

	RFLP Marker	21 G25	22 G26	23 G27	24 G28	25 G29	26 G30	27 G31	28 G32	29 G33	RILs 30 G34	31 G35	32 G36	33 G37	34 G38	35 G39	36 G40	37 G41	38 G43	39 G44	40 G45
1	umc39	A	A	B	A	B	B	A	B	B	H	A	B	A	A	B	A	A	A	B	A
2	bnl8.29	B	A	A	A	A	A	B	A	A	H	A	B	B	A	B	B	B	A	A	A
3	bnl8.29b	A	A	A	A	A	A	A	A	A	B	A	B	A	A	A	A	B	B	A	A
4	npi285	A	B	A	B	A	B	A	-	A	A	B	B	-	B	A	A	B	B	B	A
5	umc5	A	-	A	A	A	-	B	B	-	-	-	-	A	A	A	B	-	-	-	A
6	umc32	B	A	B	A	B	B	A	B	B	B	B	B	A	A	B	A	B	A	A	A
7	umc89	A	B	B	B	A	B	A	B	A	A	A	B	A	A	A	A	B	A	B	A
8	bnl6.32	B	A	A	B	B	A	B	A	A	B	A	B	B	B	B	B	B	A	A	A
9	umc200	A	A	A	A	A	B	A	A	B	A	B	B	A	A	A	A	B	B	B	A
10	umc21	B	A	B	B	B	B	A	B	B	B	A	B	B	B	B	A	B	A	A	B
11	umc107	B	A	B	A	B	B	A	A	A	A	A	B	B	A	A	B	B	A	A	B
12	umc167	-	A	A	B	-	A	A	-	-	-	-	B	-	B	A	-	B	B	B	A
		C				C						C		C			C				
13	npi268	A	A	A	A	B	B	A	A	A	A	B	B	B	B	B	B	A	B	A	A
14	npi268b	A	B	B	B	A	B	A	A	A	B	B	B	A	B	A	A	B	A	A	A
15	umc11	B	A	A	B	B	B	A	B	A	H	A	H	B	A	A	B	A	B	B	B
16	php06005	B	B	A	B	H	B	B	H	A	B	B	A	A	B	B	B	B	B	H	B
17	umc170	A	A	B	B	B	B	A	A	A	A	B	H	B	B	B	A	A	A	A	B
18	php20855	A	A	A	A	B	A	A	B	A	A	A	A	B	B	A	B	B	A	A	A
19	umc68	A	A	B	B	A	B	B	B	A	H	B	B	A	H	A	A	B	B	A	B
20	umc204	A	B	A	B	B	B	B	A	A	H	A	B	A	B	B	B	B	A	A	A
21	umc2	B	A	B	A	B	B	A	A	A	H	A	B	B	B	B	B	B	B	B	B
22	umc147	A	A	A	B	B	A	B	B	A	H	A	B	B	A	B	B	B	B	H	B
23	umc147b	B	A	B	A	A	A	A	A	A	A	A	B	-	B	A	-	B	A	A	A
24	umc16	-	B	A	B	A	B	A	A	A	B	A	B	A	A	A	A	B	A	B	A
25	umc16b	B	A	B	A	B	A	A	B	A	A	B	B	B	B	B	B	A	A	A	B
		C																			
26	umc193	A	H	A	H	B	B	A	B	H	H	B	B	B	B	B	B	A	H	H	A
27	umc193b	H	B	H	B	H	B	H	B	H	B	H	H	H	H	A	B	H	-	B	B
		C				C					C			C			C				
28	bnl12.06	B	A	A	B	B	B	A	B	B	B	A	B	B	A	A	B	B	B	A	B
			C					C								C					
29	umc67	-	A	A	A	-	A	A	-	H	H	A	B	-	B	A	-	B	B	B	A
		C				C			C					C			C				
30	php10017	A	A	B	A	A	B	-	-	B	H	B	B	A	B	A	A	B	A	B	-
31	umc51	B	A	A	B	B	A	B	B	B	H	B	B	B	B	B	A	A	A	A	B
		C				C			C		C			C			C				C
32	umc104	A	A	B	A	A	B	B	B	B	H	B	B	A	B	A	A	B	A	B	B
33	umc173	A	A	A	B	B	B	A	B	B	H	A	H	B	B	B	A	B	B	A	B
34	umc173b	A	A	B	A	B	B	B	B	A	B	A	B	B	B	B	B	A	B	B	B
35	umc63	-	B	A	B	A	B	A	-	A	-	A	B	A	A	A	-	B	A	B	A
		C							C		C						C				
36	umc140	B	A	-	A	B	A	A	A	A	H	A	A	B	A	A	B	-	A	A	B
				C														C			
37	umc128	A	A	A	A	B	A	A	A	A	A	A	A	A	B	A	B	B	B	A	B
38	php20608	B	A	A	A	A	B	B	B	A	A	A	A	B	B	B	A	B	A	B	B
39	php20608	B	B	A	B	A	A	A	B	B	B	A	A	B	B	B	B	A	B	A	A
40	umc96	B	B	A	B	A	B	A	B	A	A	A	B	A	A	A	A	B	A	B	A
41	npi107	A	A	B	A	B	B	A	B	A	H	A	B	A	A	B	A	A	A	B	A
42	umc26	A	B	B	B	A	B	A	A	B	H	B	B	A	A	A	A	B	B	A	A

Appendix 3. Cont.

	RFLP Marker	21 G25	22 G26	23 G27	24 G28	25 G29	26 G30	27 G31	28 G32	29 G33	RILs 30 G34	31 G35	32 G36	33 G37	34 G38	35 G39	36 G40	37 G41	38 G43	39 G44	40 G45
43	umc26b	A	B	A	B	A	B	A	A	B	B	B	A	A	A	A	A	A	B	B	B
44	umc131	A	B	B	B	A	B	A	A	B	H	B	H	A	B	A	A	B	B	B	A
45	umc134	A	A	A	B	A	A	A	A	B	H	A	B	A	B	A	A	B	B	B	A
46	umc103	A	A	B	A	B	B	A	B	A	H	A	B	A	A	B	A	A	A	B	A
47	umc122	A	B	B	B	A	B	A	A	B	A	B	A	A	A	A	A	B	A	B	A
																C					
48	bnl5.71	B	B	A	B	A	A	A	B	B	B	B	A	B	B	B	A	B	B	A	A
49	umc102	A	B	A	B	A	B	A	A	B	H	B	A	A	A	B	H	A	B	A	A
50	npi262	A	A	A	B	A	B	A	A	A	A	A	B	A	B	A	A	B	H	B	A
51	umc53	A	B	A	A	-	A	A	A	A	H	B	A	-	B	-	A	-	A	A	-
						C								C		C		C			C
52	bnl6.25	-	A	A	A	-	B	-	B	A	A	A	A	-	B	-	A	B	A	B	-
		C				C		C						C		C					C
53	npi238	-	A	B	A	A	A	-	A	A	A	A	B	-	A	-	-	B	B	A	A
		C						C						C		C	C				
54	umc55	A	B	B	B	B	B	B	B	B	B	B	B	A	A	B	B	B	B	B	A
55	umc133	A	-	A	B	B	A	A	B	-	B	-	B	B	A	B	B	-	B	-	B
			C							C		C						C		C	
56	umc58	A	A	A	A	B	A	A	B	A	A	A	A	B	B	A	B	B	A	A	A
57	umc121	B	B	B	A	A	A	A	B	B	B	B	A	B	A	B	A	A	A	A	A
58	umc27	B	A	A	A	A	A	A	A	A	A	A	B	B	A	A	A	A	A	B	B
59	umc120	B	A	B	A	B	B	A	B	A	B	A	B	B	B	B	B	A	B	A	B
60	umc44	A	B	B	B	A	B	B	B	A	H	B	A	A	B	A	B	B	B	A	B
61	bnl8.39	A	B	A	B	A	A	A	A	B	H	B	B	A	A	A	A	B	A	B	A
62	umc62	-	A	A	B	A	A	-	-	A	H	A	B	A	B	A	A	B	B	B	-
								C	C												C
63	bnl5.37	B	B	B	B	A	B	A	A	B	B	B	B	A	A	A	A	B	H	A	A
		C																			
64	umc185	B	A	A	B	B	B	A	B	A	A	A	A	B	A	A	B	A	B	A	B
65	bnl8.23	A	B	B	B	B	B	A	A	A	A	A	A	A	B	A	A	B	A	B	A
66	bnl7.50	A	B	B	B	-	A	-	A	B	B	B	A	-	B	-	-	B	B	B	-
						C		C						C		C	C				C
67	php20024	-	B	B	A	-	A	A	-	B	B	B	A	A	A	-	A	A	B	A	-
						C			C							C					C
68	umc124	B	H	A	B	B	A	A	B	B	H	A	A	B	B	B	B	B	H	A	B
69	umc84	B	A	A	B	B	A	B	A	A	H	B	B	B	B	B	B	B	A	A	A
70	npi286	A	A	A	B	B	B	A	B	A	B	A	B	B	A	A	B	A	B	A	B
71	umc82	B	B	A	B	A	B	A	A	A	B	A	B	B	A	A	A	B	B	A	A
72	umc82b	A	A	A	A	A	B	B	A	A	H	B	B	A	B	B	A	A	A	A	A
73	npi97	A	B	B	B	A	B	A	B	A	B	A	A	A	A	A	B	A	B	B	B
74	umc35	A	B	A	B	B	A	A	A	B	H	B	B	A	A	B	B	B	A	A	A
75	umc36	B	-	-	-	-	-	B	B	-	A	B	-	-	-	A	-	-	A	-	-
76	csu173	A	-	-	-	-	-	H	A	-	-	-	-	-	-	-	-	-	B	H	B
77	npi414	A	A	A	A	B	B	A	B	A	B	B	B	B	B	B	B	A	A	A	A
78	umc198	A	B	A	A	A	A	A	A	B	A	B	B	A	A	A	A	B	A	A	A
79	umc198b	-	A	B	B	A	A	A	-	A	A	B	A	-	B	A	-	B	A	B	-
		C							C					C			C				C
80	npi400	B	A	A	A	A	A	A	B	A	A	A	B	B	A	B	B	A	A	A	A
81	umc81	H	B	B	B	B	B	B	A	A	H	B	H	B	B	B	B	B	A	B	B
82	umc117	B	A	B	B	B	B	A	B	A	A	B	B	B	B	B	B	A	B	A	B
83	umc113	A	B	A	B	B	B	A	A	A	H	A	B	A	B	A	A	B	A	A	A
84	bnl5.59	A	B	A	A	A	B	B	A	A	A	A	B	A	B	B	A	B	B	A	A
85	umc157	H	H	A	B	A	H	H	B	A	B	A	A	B	A	A	B	A	H	B	B

Appendix 3. Cont.

	RFLP Marker	21 G25	22 G26	23 G27	24 G28	25 G29	26 G30	27 G31	28 G32	29 G33	RILs 30 G34	31 G35	32 G36	33 G37	34 G38	35 G39	36 G40	37 G41	38 G43	39 G44	40 G45
86	bnl5.09	A	B	B	B	A	A	A	A	B	A	B	B	A	A	A	A	B	B	B	A
		C				C		C						C		C	C				C
87	umc54	B	B	A	B	A	B	A	B	A	B	B	A	B	B	B	A	B	B	A	B
88	umc13	B	B	A	B	A	A	A	B	A	H	B	A	B	A	B	B	A	A	B	A
89	umc156	A	A	A	B	A	B	A	A	B	H	B	B	A	A	A	A	B	B	B	A
90	umc38	B	A	A	B	B	B	A	B	A	B	A	B	A	B	A	A	B	B	A	B
91	umc190	-	B	B	B	A	A	A	A	B	H	B	H	A	B	A	A	B	B	B	A
92	csu86	A	A	A	A	B	A	A	B	B	A	A	A	B	B	A	B	B	A	A	A
93	bnl1.297	A	B	A	B	A	B	A	A	A	H	A	B	A	A	A	A	B	A	B	A
94	umc95	B	B	B	B	B	A	B	A	B	B	B	H	B	B	B	B	B	B	B	B
95	php20725	A	A	A	B	A	A	A	A	B	A	A	B	A	A	A	A	A	B	B	A
96	csu16	A	B	B	A	A	A	A	A	B	H	B	A	A	A	A	A	A	B	A	A
97	umc72	A	A	A	A	-	A	A	-	A	A	A	A	-	B	A	-	B	A	A	A
						C			C					C			C				
98	umc43	-	A	A	B	A	A	-	-	B	H	A	B	-	B	-	A	B	B	A	A
		C						C	C					C		C					
99	umc50	A	B	A	B	A	A	A	A	B	H	B	A	A	A	-	A	A	B	B	-
																C	C				C
100	npi291	-	B	B	B	-	A	-	A	B	H	B	B	-	B	-	-	B	B	B	-
		C				C		C						C		C	C				C
101	npi291b	A	B	B	B	A	B	A	B	B	A	B	B	A	A	A	A	B	B	B	A
102	csu59	B	B	B	B	B	A	B	A	B	A	A	B	B	B	B	B	B	B	B	B
103	umc15	-	A	A	B	A	A	A	-	A	A	B	A	-	B	A	-	B	A	B	-
		C							C					C			C				C
104	umc110	A	A	A	B	A	A	H	A	A	A	A	A	A	A	A	B	A	A	A	B
105	csu133	A	B	B	B	B	B	A	B	B	A	A	B	B	A	B	A	B	B	B	A
106	php20581	A	B	A	B	A	B	B	B	B	B	B	A	A	A	A	A	B	A	A	A
107	php20581b	B	A	A	A	A	B	B	B	B	A	A	B	B	A	B	B	A	A	B	A
108	npi105	-	B	A	B	-	B	-	-	B	B	B	A	-	-	A	B	-	B	B	B
		C				C		C	C					C	C			C			
109	npi287	A	B	A	A	B	B	A	B	B	H	B	A	B	B	B	A	B	H	B	H
110	php20508	A	B	B	B	A	B	A	B	B	H	B	B	A	A	B	B	B	B	A	A
111	UMC109	A	B	A	A	-	B	B	A	B	B	B	A	A	B	B	A	A	A	A	A
112	bnl16.06	-	A	A	B	-	A	A	-	A	A	B	B	A	A	-	-	B	A	B	-
		C				C			C							C	C				C
113	bnl16.06b	B	B	B	B	B	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B
114	umc136	A	A	B	B	A	A	A	A	A	A	B	A	A	A	A	B	A	A	A	B
115	bnl14.07	A	B	A	B	B	O	A	B	B	B	B	B	A	A	B	B	A	A	B	B
116	csu50	A	B	B	B	A	B	A	A	B	H	B	B	A	B	A	A	B	B	B	A
117	php20075	A	B	A	B	A	A	A	A	A	A	B	A	A	B	A	A	B	B	A	A
118	umc132	-	A	A	B	A	B	A	A	B	A	A	B	A	B	A	A	B	B	A	A
119	umc19	A	A	A	B	A	A	A	A	A	A	B	A	A	A	A	A	B	B	A	A
120	npi393	B	A	A	B	B	A	B	B	A	B	B	-	-	A	B	A	B	A	A	-
121	umc123	-	A	A	A	A	A	A	A	A	A	A	B	A	B	A	B	B	B	B	A
122	csu30	A	B	B	B	A	B	A	B	B	A	B	B	A	A	B	B	B	B	A	A
123	npi239	-	A	A	A	A	A	-	A	A	B	B	A	-	B	-	A	B	A	B	-
		C						C			C			C		C					C
124	umc103	A	A	B	A	A	B	A	A	B	H	A	B	A	B	A	A	A	B	B	A
125	umc164	A	B	A	B	A	B	A	A	A	B	A	A	A	A	A	B	A	B	B	B
126	npi321	A	B	B	B	B	B	B	B	A	A	B	B	A	A	A	B	B	A	B	B
127	npi114	A	B	A	B	A	B	A	A	B	A	B	A	A	A	A	A	A	B	B	B

Appendix 3. Cont.

	RFLP Marker	41 G46	42 G47	43 G48	44 G49	45 G50	46 G51	47 G52	48 G53	49 G54	RILs 50 G55	51 G56	52 G57	53 G58	54 G59	55 G60	56 G62	57 G63	58 G64	59 G65	60 G66
1	umc39	A	B	A	B	A	A	A	A	A	B	H	A	B	A	A	B	B	B	A	A
2	bnl8.29	A	B	A	B	B	B	A	B	A	B	A	A	A	A	B	B	A	B	A	B
3	bnl8.29b	A	A	A	A	B	-	A	A	A	A	A	A	B	B	A	A	A	A	A	A
4	npi285	A	A	B	A	A	A	A	A	B	A	A	A	A	A	B	A	A	A	A	A
5	umc5	B	A	-	-	A	-	B	B	A	B	B	B	-	-	-	B	A	B	A	B
6	umc32	A	B	O	A	B	A	B	B	A	B	B	B	B	B	B	B	A	B	A	B
7	umc89	B	B	A	A	A	A	B	A	B	A	A	B	A	A	B	B	A	B	A	A
8	bnl6.32	B	B	A	B	B	B	A	B	B	B	A	A	A	A	H	B	A	B	B	A
9	umc200	A	A	B	B	B	B	A	A	B	A	A	A	A	B	A	A	A	A	A	A
10	umc21	B	B	B	B	A	A	B	B	B	B	B	B	B	B	B	H	A	A	B	A
11	umc107	A	A	A	B	B	B	A	B	B	A	A	A	A	A	B	B	A	B	B	B
12	umc167	A	A	A	B	H	B	A	A	A	A	- C	A	B	B	A	A	A	A	A	- C
13	npi268	B	B	B	B	B	A	B	A	A	B	A	B	A	H	B	H	B	B	A	A
14	npi268b	A	A	B	B	A	A	A	A	A	A	B	A	B	B	B	B	A	B	B	B
15	umc11	A	A	A	A	B	A	B	A	H	A	B	B	A	A	A	B	A	A	A	A
16	php06005	B	A	A	A	A	A	H	B	A	B	A	A	B	A	B	B	A	A	A	B
17	umc170	B	B	B	B	A	A	B	B	B	B	B	B	B	B	A	A	A	A	B	A
18	php20855	A	A	A	B	B	A	A	B	A	B	B	B	A	A	B	B	A	B	B	B
19	umc68	A	B	A	A	A	-	A	B	B	A	B	A	B	B	A	A	A	A	B	B
20	umc204	A	A	B	B	B	B	B	B	B	B	B	A	B	B	B	B	A	B	B	B
21	umc2	B	B	B	B	A	A	A	B	A	B	B	B	A	A	B	B	B	B	B	A
22	umc147	A	A	A	A	A	B	A	B	A	A	B	A	B	A	B	B	A	A	B	B
23	umc147b	A	-	A	A	B	B	A	-	B	-	A	A	A	A	B	-	A	B	B	B
24	umc16	A	-	B	B	A	B	-	A	A	A	A	-	B	A	B	-	A	-	-	-
25	umc16b	B	A C	B	B	B	A	B C	A	A	B	B	B C	A	A	A	B C	A	B C	B C	A C
26	umc193	B	B	B	B	B	A	B	A	A	B	A	B	B	B	B	B	B	B	A	A
27	umc193b	H	-	H	H	A	B	B	H C	B	H	B	B	B	H	H	H C	H	B	B	H
28	bnl12.06	A	A	A	A	B	A	B	B	B	A	A	B	A	B	A	A	A	A	A	B
29	umc67	A	A	A	B	A	B	A	A	A	A	- C	A	B	A	A	A	A	- C	A	- C
30	php10017	-	H	B	A	A	B	H	A	B	A	-	A	A	A	B	-	A	A	-	A
31	umc51	B	B	B	A	A	B	B C	B C	B	A	A	B	A	B	B	A	A	A	H	A C
32	umc104	A	B	B	A	A	-	B	A	B	A	B	A	A	A	B	B	A	A	B	A
33	umc173	A	B	B	B	A	-	B	B	B	B	B	B	A	B	A	A	B	A	A	A
34	umc173b	B	B	B	A	A	-	A	A	A	B	B	B	A	A	B	H	B	A	B	A
35	umc63	A	- C	A	A	A	-	- C	A	A	A	A	- C	A	A	B	- C	A	A	A	A
36	umc140	A	A	A	- C	- C	- C	A	B	A	A	A	A	A	A	- C	B	A	B	B	B
37	umc128	A	B	A	A	B	A	A	B	B	B	B	A	A	A	B	B	A	B	B	B
38	php20608	B	A	B	B	B	A	A	B	B	B	A	A	A	B	B	A	A	A	A	B
39	php20608	B	B	A	B	B	A	A	B	A	B	A	A	B	B	B	A	B	A	B	B
40	umc96	A	B	A	A	A	A	B	A	A	A	A	B	A	A	B	B	A	A	A	A
41	npi107	A	B	A	B	A	H	A	A	A	B	A	A	B	A	A	B	B	B	A	A
42	umc26	A	A	B	B	A	A	A	A	B	A	A	A	B	B	B	A	A	A	A	A

Appendix 3. Cont.

	RFLP Marker	41 G46	42 G47	43 G48	44 G49	45 G50	46 G51	47 G52	48 G53	49 G54	50 G55	51 G56	52 G57	53 G58	54 G59	55 G60	56 G62	57 G63	58 G64	59 G65	60 G66
											RILs										
43	umc26b	B	A	A	A	A	B	B	A	A	A	A	B	B	B	B	B	A	B	B	B
44	umc131	A	A	A	B	B	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A
45	umc134	A	A	B	A	A	A	A	A	B	A	A	A	B	A	A	A	A	A	A	A
46	umc103	A	B	A	B	A	A	A	A	A	B	A	A	B	A	A	B	B	B	A	A
47	umc122	A	A	B	B	A	B	A	A	B	A	A	A	A	B	A	A	A	A	A	A
								C				C									
48	bnl5.71	B	B	A	B	A	A	A	B	A	B	A	A	B	B	A	A	A	A	H	B
49	umc102	A	A	B	A	A	B	B	A	B	A	A	B	B	B	B	B	A	B	B	B
50	npi262	A	A	A	B	A	B	A	A	A	A	A	A	B	B	A	A	A	A	A	A
51	umc53	A	A	B	B	B	-	-	-	A	A	A	-	A	B	B	A	-	-	-	-
								C	C				C					C	C	C	C
52	bnl6.25	-	A	B	B	B	A	A	-	-	-	A	A	A	B	B	A	A	A	A	-
		C							C	C	C										C
53	npi238	A	-	A	A	B	-	A	-	A	A	A	A	A	A	B	-	A	-	A	-
			C						C								C		C		C
54	umc55	A	B	A	B	A	A	B	B	B	B	B	A	B	B	B	B	B	B	A	B
55	umc133	B	A	A	A	-	-	A	B	B	B	A	A	-	B	A	A	B	B	A	A
						C	C							C							
56	umc58	A	A	A	B	B	-	A	A	A	B	B	B	A	A	A	B	A	B	A	B
57	umc121	B	B	A	B	B	B	B	B	B	A	B	B	B	B	B	B	A	B	A	B
58	umc27	A	A	A	B	B	B	A	B	A	A	A	B	A	A	A	A	B	A	B	B
59	umc120	B	B	B	A	B	-	A	B	A	B	B	A	B	A	B	B	A	B	B	A
60	umc44	A	B	A	A	B	B	A	B	B	A	B	B	B	A	B	B	A	B	A	B
61	bnl8.39	A	A	-	A	B	A	A	A	A	A	A	A	B	B	H	A	A	A	A	A
62	umc62	A	-	B	B	A	A	-	A	B	-	-	H	B	B	A	-	-	A	-	A
			C					C			C	C					C	C		C	
63	bnl5.37	A	A	B	B	A	A	A	A	A	A	A	A	B	B	B	B	A	B	B	B
																	C		C	C	C
64	umc185	A	A	A	A	B	-	B	A	B	A	A	-	A	B	A	A	A	A	A	B
65	bnl8.23	A	A	B	B	A	A	A	A	B	A	A	A	A	B	B	A	A	A	A	A
66	bnl7.50	-	A	-	B	B	B	-	A	A	-	A	-	B	A	B	-	A	A	-	A
		C		C				C			C		C				C			C	
67	php20024	A	A	B	H	B	B	-	-	B	A	A	-	B	B	B	-	A	-	-	-
								C	C				C				C		C	C	C
68	umc124	H	B	A	A	A	-	A	B	A	B	B	A	B	B	A	A	B	B	B	B
69	umc84	A	B	A	B	B	B	A	B	A	B	A	A	A	A	A	B	A	B	A	A
70	npi286	A	A	-	A	B	A	B	A	B	A	A	B	H	B	A	A	A	A	A	B
71	umc82	A	A	B	A	A	A	A	A	A	A	A	A	B	A	B	B	A	B	B	B
72	umc82b	B	B	A	B	B	A	A	A	A	A	A	A	B	B	B	A	A	B	A	A
73	npi97	A	B	A	A	A	B	B	A	B	B	B	B	B	A	B	A	A	B	B	H
74	umc35	B	A	A	A	B	A	B	B	B	H	A	A	A	B	A	A	B	B	A	A
75	umc36	-	A	A	A	A	A	-	A	B	B	A	B	A	-	-	A	A	B	B	A
76	csu173	B	A	A	B	H	A	-	A	A	A	A	A	B	-	-	A	A	A	A	A
77	npi414	B	B	A	B	B	A	B	A	A	B	A	B	B	B	B	B	B	B	A	A
78	umc198	A	A	B	B	A	-	A	A	B	A	A	A	A	A	A	A	A	A	A	A
79	umc198b	-	A	B	B	B	-	A	A	B	-	A	A	A	A	A	A	-	B	A	-
		C									C							C			C
80	npi400	B	B	A	A	A	A	B	A	B	B	B	B	H	B	B	B	A	B	B	A
81	umc81	A	A	A	A	A	B	B	A	A	B	H	A	A	A	B	A	A	A	A	B
82	umc117	B	A	B	B	B	A	B	A	A	B	B	B	A	A	A	A	A	B	A	B
83	umc113	A	A	B	B	B	B	A	A	B	A	A	A	B	B	B	A	A	A	A	A
84	bnl5.59	A	A	A	B	B	B	B	A	A	A	B	A	B	B	B	A	A	A	A	A
85	umc157	A	H	A	A	A	B	H	A	A	A	B	A	B	A	B	A	A	B	B	A

Appendix 3. Cont.

	RFLP Marker	41 G46	42 G47	43 G48	44 G49	45 G50	46 G51	47 G52	48 G53	49 G54	RILs 50 G55	51 G56	52 G57	53 G58	54 G59	55 G60	56 G62	57 G63	58 G64	59 G65	60 G66
86	bnl5.09	A	A	B	B	B	-	A	A	A	A	A	A	A	A	B	A	A	A	A	A
		C	C					C	C			C	C				C	C	C	C	C
87	umc54	B	B	A	A	A	A	A	B	A	B	B	A	B	B	A	A	B	A	B	B
88	umc13	B	B	A	A	H	A	B	A	B	B	B	B	B	B	B	B	B	B	A	A
89	umc156	A	A	B	B	B	A	A	A	B	A	A	A	B	B	A	A	A	A	A	A
90	umc38	A	B	B	B	A	B	B	B	B	A	A	B	B	A	A	A	B	B	B	A
91	umc190	A	A	-	H	B	B	A	A	A	A	A	A	B	B	B	A	A	A	A	A
92	csu86	A	A	A	B	B	A	A	B	A	B	B	B	A	A	B	B	A	B	B	B
93	bnl1.297	A	A	A	B	A	B	A	A	A	A	A	A	B	A	B	A	A	A	A	A
94	umc95	B	A	A	H	B	B	B	A	A	B	A	B	B	A	B	B	A	A	H	A
95	php20725	A	A	B	B	B	A	A	A	B	A	A	A	A	B	H	A	A	A	A	A
96	csu16	A	A	B	A	B	B	B	A	B	A	A	A	B	B	B	A	A	A	A	A
97	umc72	A	A	A	B	B	A	A	A	A	-	-	-	A	A	A	-	A	-	A	-
											C	C	C				C		C		C
98	umc43	-	A	A	A	B	A	-	-	A	-	-	A	B	B	A	A	-	A	-	-
		C						C	C		C	C						C		C	C
99	umc50	-	A	B	A	A	B	-	-	A	A	A	-	B	B	B	B	A	-	-	-
		C						C	C				C				C		C	C	C
100	npi291	-	B	B	B	A	-	-	-	B	A	-	-	B	B	B	A	-	-	-	-
		C						C	C			C	C					C	C	C	C
101	npi291b	A	A	B	A	B	-	A	A	A	A	A	A	A	A	B	B	A	A	A	A
102	csu59	B	A	A	B	B	-	B	B	A	B	B	B	A	A	B	B	A	B	B	B
103	umc15	-	A	B	A	A	A	A	A	B	-	A	A	A	A	A	A	-	B	A	-
		C									C							C			C
104	umc110	B	B	A	A	A	A	A	A	A	B	B	A	A	B	A	A	A	A	A	A
105	csu133	B	B	A	B	B	B	B	B	A	A	A	B	B	B	B	B	B	A	B	B
106	php20581	A	A	A	A	A	A	A	A	A	B	A	B	A	A	A	A	A	B	B	A
107	php20581b	B	B	B	A	A	A	B	A	B	B	B	A	B	B	B	B	A	B	B	A
108	npi105	-	-	B	-	-	B	-	-	B	-	A	B	B	B	B	B	B	B	B	B
		C	C		C	C		C	C		C										
109	npi287	A	B	A	B	B	-	B	B	A	A	A	B	A	A	B	A	A	B	A	A
110	php20508	A	A	B	B	A	-	B	A	B	A	A	A	B	B	B	B	B	B	A	B
111	UMC109	A	B	B	A	B	B	A	A	B	B	A	A	B	A	B	B	A	B	B	B
112	bnl16.06	-	A	A	H	B	A	-	-	B	-	A	A	A	B	A	A	-	A	A	A
		C						C	C		C							C			
113	bnl16.06b	B	B	B	B	A	B	B	B	B	A	B	B	B	B	B	A	A	B	A	A
114	umc136	B	B	B	A	A	A	B	B	A	B	A	B	B	B	A	B	A	B	A	A
115	bnl14.07	B	A	A	A	B	A	B	B	A	A	B	B	B	A	A	A	A	H	A	A
116	csu50	A	A	-	B	B	-	A	A	A	A	A	A	B	B	B	A	A	A	A	A
117	php20075	A	A	A	A	B	B	A	A	A	A	A	A	A	A	B	A	A	A	A	A
118	umc132	A	A	-	B	A	A	A	A	B	A	A	A	B	A	A	A	A	A	A	A
119	umc19	A	A	B	A	B	B	A	A	B	A	A	A	A	B	A	A	A	A	A	A
120	npi393	A	B	B	A	A	A	A	B	B	A	A	B	B	A	B	A	A	A	A	A
121	umc123	A	B	-	B	A	A	A	A	B	B	B	B	B	B	A	A	A	B	A	A
122	csu30	A	A	B	B	A	A	B	A	B	A	A	A	B	B	B	B	B	B	A	B
123	npi239	A	A	B	B	B	B	-	-	A	A	A	-	B	A	B	A	A	-	-	-
								C	C				C						C	C	C
124	umc103	A	A	B	A	B	-	A	A	A	A	A	A	B	A	B	A	A	A	A	A
125	umc164	A	B	A	A	A	B	B	A	B	A	B	A	B	A	A	A	A	A	B	A
126	npi321	A	B	A	A	B	B	A	B	B	B	B	A	A	B	B	B	B	B	A	B
127	npi114	B	A	A	A	A	B	B	A	A	A	A	B	B	B	B	B	A	B	B	B

Appendix 3. Cont.

	RFLP Marker	61 G67	62 G68	63 G69	64 G70	65 G71	66 G72	67 G73	68 G74	69 G75	RILs 70 G76	71 G77	72 G78	73 G79	74 G80	75 G82	76 G83	77 G84	78 G85	79 G86	80 G87
1	umc39	B	B	A	B	B	A	B	B	B	A	B	B	A	A	B	A	A	A	B	B
2	bnl8.29	B	B	A	A	A	A	B	A	B	A	A	B	B	A	A	A	A	A	B	A
3	bnl8.29b	A	B	A	A	-	A	A	B	A	A	A	A	A	-	A	A	A	A	A	A
4	npi285	A	A	B	B	A	A	A	B	-	-	A	A	B	A	A	A	A	A	A	A
5	umc5	A	A	A	A	-	A	A	-	A	B	A	-	B	-	A	A	B	A	B	B
6	umc32	O	A	B	A	B	A	A	A	A	B	B	A	A	-	A	A	A	B	B	A
7	umc89	B	B	A	B	B	B	B	A	A	A	B	A	B	-	A	B	A	B	A	A
8	bnl6.32	A	A	B	A	A	A	A	A	A	B	A	B	B	-	B	A	A	A	B	A
9	umc200	A	A	B	B	A	A	A	B	A	A	A	A	A	-	A	A	A	A	A	A
10	umc21	B	A	B	A	A	A	B	B	B	A	B	A	B	-	B	A	A	B	A	A
11	umc107	B	A	A	A	B	A	B	A	B	B	B	B	A	-	A	A	A	A	B	A
12	umc167	A	A	A	A	A	-	B	B	B	A	A	-	-	-	-	-	A	A	A	A
							C						C	C		C	C				
13	npi268	B	A	A	A	A	A	A	B	A	H	B	A	A	-	B	A	A	B	B	B
14	npi268b	A	B	B	B	B	B	B	A	B	H	A	A	A	-	B	B	A	B	A	A
15	umc11	B	A	A	A	B	B	A	A	A	B	B	B	A	-	B	B	B	A	B	B
16	php06005	B	A	A	B	A	B	A	A	B	B	B	B	A	-	A	B	B	A	A	B
17	umc170	B	A	A	A	A	A	B	B	B	A	B	A	B	-	B	A	A	B	A	A
18	php20855	A	B	A	A	A	B	B	A	B	B	A	A	B	-	A	B	A	A	B	A
19	umc68	H	H	B	A	-	A	A	A	B	B	A	B	H	-	B	A	B	B	B	A
20	umc204	A	A	A	B	B	A	B	B	A	A	A	A	A	A	A	A	-	B	A	B
21	umc2	B	A	B	B	A	A	A	A	B	H	A	B	B	-	B	A	A	B	B	A
22	umc147	A	A	A	B	A	A	B	A	A	B	B	B	B	-	B	A	B	A	A	A
23	umc147b	B	B	A	A	-	A	B	A	B	-	A	-	A	-	A	A	A	A	-	A
24	umc16	-	B	B	B	A	A	B	B	B	A	A	A	-	-	A	A	A	-	A	A
25	umc16b	A	A	B	A	A	A	A	A	A	B	A	A	H	-	B	A	A	A	B	B
		C												C	C				C		
26	umc193	B	A	B	A	A	A	A	B	B	H	B	A	A	-	B	A	A	B	B	B
27	umc193b	O	B	A	H	H	H	O	H	B	H	B	O	O	-	H	H	B	H	B	B
28	bnl12.06	H	A	A	A	A	H	B	A	A	B	B	B	B	-	A	B	A	A	B	B
		C				C		C									C				
29	umc67	A	A	A	A	A	-	B	A	B	-	-	A	-	-	-	-	A	A	H	A
							C				C	C		C		C	C				
30	php10017	-	A	A	A	-	A	A	A	B	A	A	-	A	-	-	A	A	A	-	A
31	umc51	B	B	B	A	B	A	A	A	B	A	B	B	B	-	A	A	A	A	A	B
		C									C		C	C		C				C	C
32	umc104	B	A	A	A	-	A	A	A	B	B	A	B	A	-	B	A	H	A	B	A
33	umc173	B	A	A	B	-	A	B	B	B	A	B	A	B	-	B	A	A	B	A	A
34	umc173b	B	A	B	B	-	A	A	A	A	H	A	B	B	-	B	A	A	A	B	A
35	umc63	-	B	B	B	-	-	A	B	A	A	A	A	A	-	A	-	A	-	A	A
		C					C										C		C		
36	umc140	B	-	A	A	B	A	-	A	-	B	B	B	B	-	A	A	B	A	A	A
			C					C		C											
37	umc128	A	B	A	A	A	B	B	A	B	B	A	A	A	-	B	B	B	A	B	A
38	php20608	A	B	A	A	A	B	B	B	B	A	B	B	A	-	A	B	A	A	B	A
39	php20608	H	A	A	B	A	A	B	B	B	A	B	B	B	-	A	B	A	B	A	B
40	umc96	B	B	B	B	B	B	A	A	A	A	B	A	B	-	A	B	A	B	A	B
41	npi107	B	B	B	B	B	A	B	B	B	A	B	B	A	-	B	A	A	A	B	B
42	umc26	A	B	B	B	A	A	A	B	A	A	A	A	A	-	A	A	A	A	A	A

Appendix 3. Cont.

	RFLP Marker	61 G67	62 G68	63 G69	64 G70	65 G71	66 G72	67 G73	68 G74	69 G75	70 G76	71 G77	72 G78	73 G79	74 G80	75 G82	76 G83	77 G84	78 G85	79 G86	80 G87
		RILs																			
43	umc26b	A	A	A	B	A	A	B	A	A	A	B	B	A	-	A	A	-	A	A	A
44	umc131	A	B	A	B	A	A	A	B	B	A	A	A	A	-	A	A	A	A	A	A
45	umc134	A	B	B	B	A	A	A	A	B	A	A	A	A	-	A	A	A	A	A	A
46	umc103	H	B	A	B	B	A	B	B	B	A	B	B	A	-	B	A	A	A	B	B
47	umc122	A	B	A	A	A	A	B	B	A	A	A	A	A	-	A	A	A	A	A	A
		C		C																	
48	bnl5.71	A	A	A	B	A	A	B	A	B	B	B	B	A	-	A	A	B	B	A	B
49	umc102	A	A	B	B	A	A	B	A	A	A	B	A	H	-	B	A	A	B	A	A
50	npi262	A	A	A	A	A	A	B	B	B	A	A	A	A	-	A	A	A	A	A	A
51	umc53	A	A	A	A	-	B	B	B	B	A	A	A	A	-	-	A	-	A	-	-
																C		C		C	C
52	bnl6.25	A	B	A	A	A	-	-	B	B	H	B	B	A	-	A	-	A	A	-	A
							C	C									C			C	
53	npi238	-	B	A	A	-	A	B	A	B	A	A	-	A	-	A	A	A	A	-	-
		C											C							C	C
54	umc55	A	B	A	B	B	A	A	B	B	B	B	A	B	-	A	A	B	A	B	B
55	umc133	A	B	A	A	-	B	A	B	A	B	B	B	A	-	B	B	B	B	B	B
56	umc58	A	A	A	B	-	B	A	A	B	B	A	A	B	-	B	B	A	A	B	A
57	umc121	A	A	A	A	B	B	A	A	A	A	B	A	A	-	A	B	A	B	B	A
58	umc27	A	A	A	B	A	A	A	B	A	A	A	A	A	-	A	A	A	A	B	B
59	umc120	B	A	B	B	-	A	B	A	A	B	A	B	B	-	B	A	A	B	B	A
60	umc44	B	A	A	B	A	A	A	B	B	A	A	B	A	-	A	A	B	A	B	B
61	bnl8.39	A	B	A	A	A	A	A	B	A	B	A	A	A	A	B	A	A	A	A	A
62	umc62	A	B	B	B	-	A	B	A	B	A	A	-	A	A	A	A	A	-	-	-
						C							C						C	C	C
63	bnl5.37	A	B	B	A	B	A	B	B	B	A	A	A	B	B	B	B	A	B	A	A
																	C		C		
64	umc185	A	A	A	A	B	B	B	A	A	B	B	B	A	-	A	B	A	A	B	B
65	bnl8.23	A	B	A	A	A	A	A	B	B	A	A	A	A	B	A	A	A	A	A	A
66	bnl7.50	-	A	A	-	-	-	B	B	B	A	-	A	A	A	-	-	A	A	A	A
		C			C	C	C					C				C	C				
67	php20024	-	H	H	B	-	-	B	A	A	A	A	A	-	B	A	-	A	-	-	A
		C				C	C							C			C		C	C	
68	umc124	B	H	A	B	-	A	H	A	H	B	B	B	B	-	B	A	A	B	B	B
69	umc84	A	A	B	A	A	B	B	A	A	B	A	B	A	-	A	B	A	A	B	A
70	npi286	A	A	A	-	A	B	B	A	A	B	B	B	A	B	A	B	A	A	B	B
71	umc82	A	B	B	B	B	A	B	H	B	H	B	A	B	-	A	A	A	B	A	A
72	umc82b	B	A	B	A	A	A	H	B	B	A	B	A	A	-	B	A	A	B	B	B
73	npi97	B	B	A	A	A	A	B	A	B	A	B	B	B	-	A	A	B	B	B	B
74	umc35	A	H	B	A	A	B	B	B	A	B	A	B	B	-	B	B	B	A	B	A
75	umc36	A	B	-	A	B	A	-	B	A	H	-	B	A	-	H	A	B	-	B	B
76	csu173	A	A	B	A	A	A	-	A	B	A	-	B	A	-	A	A	A	-	A	B
77	npi414	B	A	B	A	A	A	A	B	B	A	B	A	A	-	B	A	A	B	B	B
78	umc198	A	B	B	A	-	A	B	A	A	A	A	A	A	-	A	A	A	A	A	A
79	umc198b	A	B	A	A	-	B	B	A	A	A	A	A	-	-	A	B	A	A	A	-
		C										C		C							C
80	npi400	A	A	B	A	A	B	A	B	A	B	A	B	B	-	A	B	H	A	B	A
81	umc81	B	A	A	B	B	A	B	B	A	A	B	A	A	-	B	A	A	B	B	A
82	umc117	B	A	B	A	A	A	A	B	A	B	A	B	B	-	B	A	A	B	B	B
83	umc113	A	A	A	B	A	A	B	B	A	A	A	A	A	-	A	A	A	A	A	A
84	bnl5.59	B	A	B	B	B	A	B	A	B	A	A	A	A	-	A	B	A	A	A	A
85	umc157	B	B	A	A	B	A	B	A	A	A	B	B	A	-	A	A	B	B	B	B

Appendix 3. Cont.

	RFLP Marker	61 G67	62 G68	63 G69	64 G70	65 G71	66 G72	67 G73	68 G74	69 G75	RILs 70 G76	71 G77	72 G78	73 G79	74 G80	75 G82	76 G83	77 G84	78 G85	79 G86	80 G87
86	bnl5.09	A	B	A	A	-	A	A	B	A	A	A	A	A	-	A	A	A	A	A	A
		C					C						C	C		C	C		C		
87	umc54	A	A	B	A	B	A	A	A	B	B	B	B	A	-	B	A	B	B	B	B
88	umc13	A	A	B	A	B	A	A	A	A	B	A	B	B	-	A	A	H	A	B	B
89	umc156	A	A	A	B	A	A	H	B	B	A	A	A	A	-	A	A	A	A	A	A
90	umc38	B	B	A	B	B	A	B	B	B	A	A	A	B	-	A	A	A	B	A	A
91	umc190	A	A	A	-	A	A	B	B	A	A	A	A	A	A	A	A	A	A	A	A
92	csu86	A	B	B	A	A	B	B	A	B	B	A	A	B	-	A	B	A	A	B	A
93	bnl1.297	A	B	B	B	A	A	B	A	B	A	A	A	A	-	A	A	A	A	A	A
94	umc95	B	A	A	B	B	B	B	B	A	H	B	A	A	-	B	B	A	A	A	A
95	php20725	A	B	B	B	A	A	A	B	B	A	A	A	A	-	A	A	A	A	A	A
96	csu16	A	H	A	B	A	A	B	A	A	A	A	A	A	-	A	A	A	A	A	A
97	umc72	A	A	A	-	A	-	A	A	B	-	A	-	A	A	-	-	A	A	-	A
							C				C		C			C	C			C	
98	umc43	A	B	A	B	-	A	B	A	B	A	-	-	A	-	A	A	-	A	A	-
						C						C	C	C				C			C
99	umc50	A	A	A	B	-	A	B	A	B	A	-	A	-	B	A	A	A	-	-	A
						C						C		C					C	C	
100	npi291	-	B	B	B	-	-	B	B	B	A	A	-	-	-	-	-	A	-	A	A
		C					C						C	C		C	C		C		
101	npi291b	A	B	A	B	-	A	B	B	A	B	B	A	A	-	A	A	B	A	A	B
102	csu59	B	A	A	B	-	B	B	B	B	A	A	A	A	-	B	B	A	B	A	A
103	umc15	A	A	A	A	-	B	B	A	A	A	A	A	-	-	A	B	A	A	A	-
						C						C		C							C
104	umc110	B	A	A	A	A	A	A	A	A	B	A	B	A	-	B	A	B	A	B	B
105	csu133	B	A	A	B	-	A	B	B	B	B	A	A	B	-	A	A	A	A	B	B
106	php20581	A	B	A	A	B	A	B	B	A	B	A	B	A	-	B	A	B	A	B	B
107	php20581b	A	A	B	A	A	A	A	B	A	B	A	B	A	-	A	A	A	A	A	A
108	npi105	-	-	A	B	-	A	B	A	-	-	B	B	-	-	A	A	-	-	A	A
		C	C			C				C	C			C				C	C		
109	npi287	H	A	A	B	-	A	B	B	B	B	A	B	B	-	A	A	B	B	B	B
110	php20508	A	A	B	B	-	B	B	B	A	A	B	A	B	-	B	B	A	B	A	A
111	UMC109	A	A	A	B	A	B	A	B	A	B	A	A	B	A	A	B	A	A	B	B
112	bnl16.06	A	B	B	A	A	A	B	B	A	-	A	-	A	-	-	A	-	A	-	-
											C		C			C		C		C	C
113	bnl16.06b	B	B	B	A	A	B	B	A	B	A	B	B	B	-	B	B	A	A	B	B
114	umc136	A	A	B	A	B	A	A	A	A	B	A	B	B	-	B	A	B	A	B	B
115	bnl14.07	H	B	A	A	A	A	B	B	A	B	B	B	A	-	B	A	B	A	B	B
116	csu50	A	B	B	B	-	A	A	B	B	A	A	A	A	-	A	A	A	A	A	A
117	php20075	A	A	B	B	A	A	H	B	A	A	A	A	A	-	A	A	A	A	A	A
118	umc132	A	B	B	-	A	A	A	B	B	A	A	A	A	A	A	A	A	A	A	A
119	umc19	A	B	A	A	A	A	B	B	A	A	A	A	A	-	A	A	A	A	A	A
120	npi393	B	A	A	A	A	A	A	A	B	A	B	B	B	-	A	A	A	B	A	A
121	umc123	A	A	A	-	B	B	B	H	A	B	B	B	B	A	B	B	B	A	A	A
122	csu30	A	A	B	A	A	B	B	B	A	A	B	A	B	-	B	B	A	A	A	A
123	npi239	A	A	A	A	A	A	H	A	B	A	-	-	-	-	-	A	-	-	-	A
												C	C	C		C		C	C	C	
124	umc103	H	A	A	A	-	A	A	B	A	A	A	A	A	-	A	A	A	A	A	A
125	umc164	B	B	A	A	A	A	B	A	B	A	B	B	A	-	A	A	B	B	B	B
126	npi321	A	A	B	A	A	A	B	B	B	B	A	B	A	-	A	A	B	A	A	B
127	npi114	A	A	A	-	A	A	B	A	A	A	B	A	B	B	A	A	A	A	A	A

Appendix 3. Cont.

	RFLP Marker	81 G88	82 G89	83 G90	84 G91	85 G92	86 G94	87 G95	88 G96	89 G97	RILs 90 G98	91 G99	92 G100	93 G101	94 G102	95 G103	96 G104	97 G105	98 G106	99 G107	100 G108
1	umc39	B	A	A	B	B	B	A	B	A	A	B	O	B	A	A	B	B	B	B	B
2	bnl8.29	H	B	B	B	H	B	B	B	B	B	A	A	B	A	B	A	A	A	A	A
3	bnl8.29b	B	A	A	A	-	B	A	A	A	-	A	A	A	A	B	B	A	B	A	A
4	npi285	A	A	A	A	A	B	A	B	A	B	A	A	A	A	B	B	A	A	A	-
5	umc5	-	A	-	B	-	-	B	A	B	-	B	B	A	B	-	-	-	-	B	B
6	umc32	A	A	B	B	A	A	A	A	A	-	B	A	A	A	B	B	A	A	B	B
7	umc89	A	B	B	B	A	B	B	A	A	-	A	B	A	B	A	A	B	B	B	B
8	bnl6.32	A	B	B	B	B	-	B	B	B	-	B	B	B	A	B	A	A	A	A	A
9	umc200	B	A	B	A	A	H	A	B	A	-	A	A	A	A	B	B	A	B	A	A
10	umc21	A	A	A	B	B	B	A	B	A	-	A	A	B	B	B	B	B	A	A	A
11	umc107	B	B	B	A	A	B	B	A	B	-	A	B	A	A	B	A	B	A	B	B
12	umc167	A	-	A	A	-	-	-	B	-	-	-	-	-	-	B	B	A	A	-	-
			C			C		C		C		C	C	C	C					C	C
13	npi268	B	H	A	B	B	B	A	A	B	-	B	A	A	A	B	B	A	B	A	A
14	npi268b	B	A	B	A	A	B	B	A	A	-	A	A	A	A	A	B	B	B	A	A
15	umc11	A	B	B	A	A	A	B	A	B	-	A	B	A	A	B	B	B	A	B	B
16	php06005	A	B	A	H	A	H	B	A	A	-	H	H	A	B	A	B	B	A	B	B
17	umc170	A	A	A	B	B	A	A	H	A	-	A	A	B	A	B	B	B	A	B	B
18	php20855	A	B	B	A	A	B	B	A	B	-	A	A	A	A	B	B	A	A	B	B
19	umc68	A	A	B	B	-	B	A	A	B	-	B	A	B	A	H	A	A	A	A	A
20	umc204	B	A	A	B	B	A	B	A	B	A	A	A	A	A	A	B	B	B	B	B
21	umc2	A	B	A	B	B	B	A	A	B	-	B	A	A	A	A	A	A	B	B	B
22	umc147	A	B	A	B	B	B	A	A	B	-	B	A	B	A	B	B	B	A	B	B
23	umc147b	A	-	B	-	-	-	-	A	-	-	A	-	-	-	B	A	-	A	A	A
24	umc16	A	A	B	A	A	B	A	A	A	-	-	-	-	-	B	B	A	A	A	A
25	umc16b	A	B	A	B	B	-	A	A	B	-	B	A	A	A	A	B	A	B	B	B
											C	C	C	C	C						
26	umc193	B	B	H	B	B	-	A	A	B	-	B	A	B	A	B	B	-	B	B	B
27	umc193b	B	B	B	H	B	-	B	A	H	-	H	B	B	H	B	H	-	H	B	B
																				C	C
28	bnl12.06	A	B	B	A	B	A	B	A	B	-	B	B	A	A	B	A	A	A	B	B
29	umc67	B	-	A	A	A	A	-	B	-	-	A	-	-	A	B	A	A	A	-	-
			C					C		C			C	C						C	C
30	php10017	A	A	A	-	-	B	A	A	H	-	-	A	-	-	A	A	A	A	A	A
31	umc51	B	A	A	B	B	A	A	B	A	-	A	B	A	A	A	B	A	B	A	A
						C		C		C		C	C	C	C					C	
32	umc104	A	A	A	B	-	B	A	A	B	-	B	A	B	B	A	A	A	A	A	A
33	umc173	A	A	A	B	-	A	A	B	A	-	A	A	B	A	B	B	B	A	B	B
34	umc173b	A	B	A	B	-	B	A	A	A	-	H	B	A	B	A	B	A	B	A	A
35	umc63	A	A	A	-	-	A	-	B	A	-	A	-	-	-	A	B	A	A	A	A
					C			C					C	C	C						
36	umc140	-	B	-	A	A	-	B	A	A	-	A	B	A	A	-	A	B	A	B	B
		C		C			C									C					
37	umc128	B	B	B	A	A	B	B	A	A	-	B	A	A	A	B	B	A	A	B	B
38	php20608	A	B	A	A	B	B	A	B	A	-	B	B	A	B	A	B	B	A	A	A
39	php20608b	B	A	B	B	B	A	H	B	B	-	A	B	B	A	B	B	B	B	B	B
40	umc96	A	B	B	B	A	A	B	A	B	-	A	B	A	B	A	A	B	A	B	B
41	npi107	B	A	A	B	B	B	A	B	B	-	B	B	B	A	A	B	B	B	A	B
42	umc26	A	A	A	A	A	A	A	B	A	-	H	A	A	A	H	B	A	B	A	A

Appendix 3. Cont.

	RFLP Marker	81 G88	82 G89	83 G90	84 G91	85 G92	86 G94	87 G95	88 G96	89 G97	RILs 90 G98	91 G99	92 G100	93 G101	94 G102	95 G103	96 G104	97 G105	98 G106	99 G107	100 G108
43	umc26b	A	B	B	A	A	A	A	A	B	-	A	B	A	B	B	B	B	B	A	A
44	umc131	B	A	B	A	A	-	A	A	A	-	A	A	A	A	B	A	-	B	A	A
45	umc134	A	A	A	A	A	B	A	A	A	-	A	A	A	A	A	B	A	A	A	A
46	umc103	B	A	A	B	B	B	A	H	B	-	B	B	B	A	A	B	H	B	B	H
47	umc122	B	A	B	A	A	B	A	A	A	-	A	A	A	A	A	B	A	A	A	A
									C					C				C			C
48	bnl5.71	B	A	B	B	B	B	A	B	A	-	B	B	B	A	B	B	B	B	B	B
49	umc102	A	A	A	A	A	A	H	A	B	-	B	A	A	B	A	B	B	B	A	A
50	npi262	A	A	A	A	A	-	A	B	A	-	A	A	A	A	B	B	A	A	A	A
51	umc53	A	A	A	A	-	-	A	A	A	-	-	A	-	A	A	A	-	A	A	A
												C		C				C			
52	bnl6.25	A	-	A	A	-	B	A	B	A	-	-	-	A	-	A	B	-	A	A	A
			C			C						C	C		C			C			
53	npi238	H	-	B	-	-	B	-	B	-	-	A	-	-	A	B	B	A	A	A	A
			C		C			C		C			C	C							
54	umc55	B	A	B	B	B	-	B	A	B	-	B	B	A	B	B	A	-	B	B	B
55	umc133	A	B	-	A	-	A	B	A	B	-	B	B	B	B	B	-	A	A	B	B
				C													C				
56	umc58	B	B	B	A	-	-	B	B	B	-	A	B	A	A	B	B	A	A	B	B
57	umc121	A	A	B	A	A	A	A	A	A	-	B	A	A	A	B	B	A	B	A	A
58	umc27	A	B	B	A	A	B	A	A	A	-	A	B	A	B	B	B	A	B	B	A
59	umc120	A	B	A	B	-	B	A	A	A	-	B	B	A	B	A	B	B	B	-	A
60	umc44	A	B	B	A	A	B	B	B	A	-	A	A	A	B	A	B	A	B	B	B
61	bnl8.39	B	A	B	-	A	A	A	A	A	B	A	-	A	A	B	B	A	A	-	A
62	umc62	A	-	A	B	-	-	A	A	-	B	A	A	B	A	A	B	-	A	-	-
			C			C	C			C		C						C			C
63	bnl5.37	B	A	A	B	A	A	B	A	A	A	A	-	A	A	A	B	A	B	-	A
		C			C			C													
64	umc185	A	B	B	A	-	B	B	A	B	-	B	-	A	A	B	B	B	A	-	B
65	bnl8.23	A	A	A	B	A	A	A	B	A	A	A	A	A	A	A	B	A	A	-	A
66	bnl7.50	A	A	A	-	-	-	-	-	B	B	-	B	-	-	A	B	B	B	A	-
					C	C	C	C	C			C		C	C						C
67	php20024	H	-	B	-	A	A	A	A	-	A	A	A	A	-	B	B	-	-	-	A
			C		C					C											
68	umc124	H	-	A	B	-	A	A	A	A	-	A	B	A	B	B	B	B	B	B	A
69	umc84	A	B	B	B	B	-	B	B	B	-	B	B	B	A	B	A	-	A	A	A
70	npi286	A	B	B	A	B	B	B	B	B	B	B	-	A	A	B	H	A	A	-	B
71	umc82	A	B	B	A	A	A	A	A	A	-	A	A	H	A	A	B	A	B	H	A
72	umc82b	B	A	A	A	A	B	A	B	A	-	A	A	A	A	A	B	A	B	A	A
73	npi97	A	B	B	A	B	A	B	A	A	-	B	B	B	H	B	A	A	A	B	B
74	umc35	A	B	B	A	B	A	B	A	B	-	A	A	A	A	A	B	A	A	B	A
75	umc36	A	B	A	A	A	-	-	B	A	-	B	B	A	A	-	-	-	A	B	-
76	csu173	B	A	B	B	A	-	H	B	A	-	-	A	-	A	-	-	-	B	A	-
77	npi414	B	B	A	B	B	B	A	B	B	-	B	A	B	A	B	B	A	B	B	B
78	umc198	B	A	A	A	-	B	A	B	A	-	A	A	A	A	B	A	A	A	A	A
79	umc198b	A	-	B	A	-	A	-	B	A	-	-	-	A	-	B	B	-	B	A	A
			C					C				C	C		C			C			
80	npi400	B	A	A	B	H	-	A	B	B	-	A	A	B	B	B	A	A	A	B	B
81	umc81	A	A	A	A	A	B	B	A	B	-	B	A	B	B	A	B	B	A	B	B
82	umc117	B	B	B	B	B	-	A	A	B	-	B	A	A	A	B	B	-	B	A	A
83	umc113	B	A	A	A	A	A	A	A	A	-	B	A	A	A	A	B	B	B	-	A
84	bnl5.59	B	A	A	B	A	B	B	B	A	-	A	A	A	A	B	B	A	A	A	A
85	umc157	A	B	B	A	B	A	H	A	A	-	A	B	H	A	B	A	A	A	B	H

Appendix 3. Cont.

	RFLP Marker	81 G88	82 G89	83 G90	84 G91	85 G92	86 G94	87 G95	88 G96	89 G97	RILs 90 G98	91 G99	92 G100	93 G101	94 G102	95 G103	96 G104	97 G105	98 G106	99 G107	100 G108
86	bnl5.09	B	A	B	A	-	B	A	A	A	-	A	A	A	A	B	B	A	B	A	A
			C		C			C		C		C		C	C			C		C	C
87	umc54	B	B	B	B	H	B	A	A	A	-	B	A	B	A	B	A	B	A	A	A
88	umc13	B	A	A	B	H	A	B	B	B	-	B	A	B	B	B	A	A	A	B	B
89	umc156	B	A	B	A	A	B	A	A	A	-	A	A	A	A	B	A	A	B	A	A
90	umc38	B	A	A	A	B	A	A	B	A	-	A	A	B	A	B	B	B	A	B	B
91	umc190	A	A	A	A	A	A	A	A	A	B	A	-	A	A	A	B	B	B	-	A
92	csu86	A	B	B	A	A	B	B	A	B	-	A	A	A	A	B	B	A	A	B	B
93	bnl1.297	A	A	B	A	A	B	A	A	A	-	A	A	A	A	B	B	A	A	A	A
94	umc95	A	A	A	B	B	B	B	A	B	-	B	A	B	B	A	B	B	B	B	B
95	php20725	B	A	B	A	A	B	A	B	A	-	A	A	A	A	A	B	A	B	A	A
96	csu16	H	A	B	A	A	A	A	A	A	-	A	A	A	A	B	B	A	B	A	A
97	umc72	B	-	B	A	A	-	-	B	-	B	A	-	A	A	B	B	A	A	-	-
			C				C	C		C			C								C
98	umc43	B	A	A	-	-	A	A	B	-	-	A	-	-	A	B	B	A	B	-	-
					C	C				C		C	C	C						C	C
99	umc50	A	A	B	A	A	-	A	A	-	A	A	-	A	A	B	B	-	B	-	A
							C			C								C			
100	npi291	B	-	B	A	-	B	-	A	-	-	A	A	-	-	B	B	-	B	-	-
			C					C		C				C	C			C		C	C
101	npi291b	A	A	B	A	-	B	A	B	A	-	A	B	A	A	B	B	A	B	A	A
102	csu59	B	B	A	B	-	B	B	A	B	-	B	A	B	B	B	B	B	B	B	B
103	umc15	A	-	B	A	A	A	-	A	A	-	-	-	A	-	B	B	-	A	A	A
			C					C				C	C		C			C			
104	umc110	B	B	A	B	A	A	B	A	B	-	A	B	B	B	A	A	A	A	B	B
105	csu133	B	B	B	B	-	B	A	B	B	-	B	A	A	A	B	A	A	B	B	B
106	php20581	A	B	A	A	A	-	A	A	A	-	A	B	A	A	B	B	-	B	B	B
107	php20581b	A	A	A	A	A	B	-	B	B	-	A	B	B	A	A	A	-	A	B	B
108	npi105	-	B	B	-	-	-	-	-	B	-	B	-	-	B	A	B	-	B	-	A
		C			C	C		C	C				C	C						C	
109	npi287	A	B	A	A	-	B	A	B	B	-	B	A	B	A	B	A	B	B	B	B
110	php20508	A	A	A	A	-	-	H	A	B	-	A	A	A	B	A	B	B	B	A	A
111	UMC109	B	A	A	B	B	A	B	A	B	B	A	A	B	A	A	B	B	A	B	B
112	bnl16.06	A	-	B	A	-	-	-	A	-	-	A	A	-	-	B	B	A	A	A	A
			C			C		C		C				C	C						
113	bnl16.06b	B	A	B	B	B	B	B	B	A	-	B	B	B	B	B	B	B	B	A	A
114	umc136	B	A	A	A	A	-	B	B	B	-	A	B	B	B	B	B	A	-	A	B
115	bnl14.07	B	B	B	A	B	A	B	A	B	-	B	B	B	B	B	B	B	A	B	B
116	csu50	B	A	B	A	-	-	A	A	A	-	A	A	A	A	B	A	A	B	A	A
117	php20075	A	A	B	A	A	B	A	B	A	-	A	A	A	A	B	B	A	B	A	A
118	umc132	A	A	A	A	A	A	A	A	A	B	A	-	A	A	A	B	B	A	-	A
119	umc19	A	A	B	A	A	B	A	A	A	-	A	A	A	A	B	B	A	B	A	A
120	npi393	A	A	A	B	B	-	A	A	A	-	A	-	-	-	B	B	-	A	A	A
121	umc123	A	A	A	B	B	A	A	-	A	B	A	-	A	-	-	-	-	-	-	-
122	csu30	A	A	A	A	A	-	B	-	B	-	A	-	A	B	A	B	B	B	A	A
123	npi239	A	A	A	-	-	-	A	B	A	-	-	A	-	-	A	A	-	A	A	A
					C	C						C		C	C						
124	umc103	B	A	A	A	-	B	A	A	A	-	A	A	A	A	A	B	A	B	A	A
125	umc164	B	B	B	A	B	A	B	A	A	-	B	B	B	A	A	A	A	A	B	B
126	npi321	A	B	B	A	A	A	B	A	A	-	B	B	B	B	B	A	A	A	A	B
127	npi114	A	B	B	-	A	A	A	A	B	A	B	B	A	B	B	B	B	B	-	A

Appendix 3. Cont.

	RFLP Marker	101 G111	102 G112	103 G114	104 G115	105 G116	106 G117	107 G118	RILs 108 G119	109 G120	110 G121	111 G122	112 G124	113 G125	114 G126	115 G127	116 G128	117 G129
1	umc39	B	B	A	B	A	B	A	B	B	B	A	A	B	B	A	B	A
2	bnl8.29	A	A	A	H	B	B	B	B	A	A	A	B	B	B	B	B	B
3	bnl8.29b	B	B	-	A	-	A	A	A	A	A	A	B	A	A	A	A	A
4	npi285	B	B	A	B	A	-	B	B	B	B	A	B	B	B	A	B	A
5	umc5	-	A	-	A	-	B	A	-	B	B	A	-	B	B	A	-	A
6	umc32	A	B	A	B	B	B	A	A	A	B	B	A	A	A	B	B	B
7	umc89	A	A	B	A	A	B	A	B	B	A	B	A	B	A	A	B	A
8	bnl6.32	A	A	A	B	B	A	A	O	A	A	A	B	A	B	H	A	B
9	umc200	A	B	A	H	A	A	A	B	A	A	A	A	A	A	A	A	A
10	umc21	B	B	B	B	B	B	A	A	B	B	B	B	A	A	A	B	B
11	umc107	A	B	B	A	B	B	B	B	A	B	B	B	B	A	B	B	A
12	umc167	B	A	-	H	-	-	A	-	-	-	A	A	A	-	A	A	-
				C						C	C				C			C
13	npi268	B	A	B	A	B	A	B	B	B	B	B	A	A	A	A	B	B
14	npi268b	B	B	B	H	A	B	A	A	A	B	A	B	B	B	H	B	B
15	umc11	B	A	B	A	A	A	A	B	B	B	B	A	B	B	A	B	B
16	php06005	A	A	A	A	B	B	B	A	A	B	A	B	A	A	B	A	A
17	umc170	B	B	B	B	B	B	A	A	B	B	B	B	A	A	A	B	B
18	php20855	B	B	B	H	B	B	B	H	B	B	A	B	B	A	A	A	A
19	umc68	A	B	-	B	-	H	B	B	B	B	A	A	A	A	B	B	A
20	umc204	A	A	B	A	A	B	A	A	A	A	B	A	A	B	A	A	A
21	umc2	B	A	B	A	A	B	B	B	B	B	A	B	B	A	A	B	A
22	umc147	A	B	B	B	B	B	A	A	B	A	A	A	B	A	B	B	B
23	umc147b	B	B	-	-	-	-	B	-	A	-	-	B	-	A	-	B	-
24	umc16	A	A	-	A	A	-	A	A	-	-	A	A	A	A	A	A	A
25	umc16b	B	B	B	H	A	H	A	B	B	B	A	A	A	A	A	B	B
				C			C			C	C							
26	umc193	B	H	B	H	A	A	B	-	-	-	A	A	A	A	A	B	A
27	umc193b	B	H	H	H	B	A	A	H	-	-	H	B	-	B	B	B	H
				C		C	C					C		C		C		C
28	bnl12.06	B	A	B	A	A	A	B	B	B	B	A	A	B	B	H	A	B
29	umc67	B	A	-	A	-	A	A	A	-	-	A	A	A	-	A	A	-
				C	C	C				C	C				C			C
30	php10017	B	A	-	H	-	-	B	B	-	A	A	A	A	A	-	B	A
31	umc51	B	B	B	A	B	B	B	A	B	B	B	A	A	B	B	B	A
				C						C	C			C	C			C
32	umc104	B	B	-	B	-	B	B	B	B	A	A	A	A	A	B	B	A
33	umc173	B	B	-	B	-	B	A	A	B	A	B	B	B	A	A	B	B
34	umc173b	B	A	-	A	-	B	B	B	B	B	B	B	B	A	H	A	A
35	umc63	B	A	-	A	-	-	A	A	A	-	-	A	A	A	A	B	A
							C				C	C						
36	umc140	-	-	B	A	B	B	-	-	A	A	B	-	B	A	B	-	A
		C	C		C			C	C				C				C	
37	umc128	B	B	B	H	B	-	B	-	A	A	B	B	B	H	A	A	A
38	php20608	A	B	B	H	B	B	B	A	B	A	A	B	A	B	B	A	A
39	php20608	A	B	B	B	B	A	B	B	B	A	B	A	B	A	A	B	B
40	umc96	A	A	B	A	A	B	A	A	B	B	B	A	B	A	A	-	A
41	npi107	B	B	A	B	A	-	A	-	B	B	-	-	B	B	A	B	A
42	umc26	H	B	A	A	A	-	A	-	A	A	-	A	A	A	A	B	A

Appendix 3. Cont.

	RFLP Marker	101 G111	102 G112	103 G114	104 G115	105 G116	106 G117	107 G118	RILs 108 G119	109 G120	110 G121	111 G122	112 G124	113 G125	114 G126	115 G127	116 G128	117 G129
43	umc26b	A	A	A	A	A	A	A	B	A	B	A	A	A	A	B	B	A
44	umc131	B	B	A	A	A	A	A	B	-	-	A	A	A	A	A	A	A
45	umc134	B	A	A	A	A	A	B	H	A	A	A	A	A	A	A	B	A
46	umc103	B	B	A	B	A	A	A	B	B	B	A	A	B	B	A	B	A
47	umc122	A	B	A	H	A	A	H	B	A	A	A	B	A	A	A	B	A
			C				C					C						
48	bnl5.71	B	B	A	B	A	A	B	B	B	B	A	A	B	A	B	B	B
49	umc102	A	B	A	A	A	A	B	B	A	A	A	A	A	A	H	B	A
50	npi262	B	A	A	A	A	-	A	-	A	A	A	A	A	A	A	A	A
51	umc53	-	B	-	B	-	A	A	A	-	A	-	B	A	-	-	B	-
		C								C		C			C	C		C
52	bnl6.25	A	B	-	H	-	-	-	A	-	A	A	B	A	-	-	A	A
				C		C	C	C		C					C	C		
53	npi238	A	B	-	-	-	-	B	B	A	-	A	B	-	A	-	B	O
					C		C				C			C		C		C
54	umc55	B	A	A	A	B	B	A	B	-	-	A	B	B	B	A	H	A
55	umc133	-	A	-	A	-	A	A	-	B	B	B	B	B	A	B	A	A
		C							C									
56	umc58	B	A	-	A	-	B	B	B	B	B	A	A	B	B	A	A	B
57	umc121	A	B	A	A	B	B	B	B	A	B	A	A	A	A	A	B	A
58	umc27	B	A	B	A	A	A	B	A	B	B	A	B	B	B	A	B	A
59	umc120	B	A	-	A	-	B	B	B	B	B	B	B	B	B	B	A	B
60	umc44	A	A	B	H	B	B	B	A	B	B	B	A	B	B	B	A	A
61	bnl8.39	B	A	A	A	A	A	A	B	A	A	A	B	B	A	A	A	A
62	umc62	B	A	A	A	-	A	B	B	-	A	-	B	A	A	-	B	A
					C	C				C		C				C		
63	bnl5.37	A	B	A	A	A	A	B	A	A	B	A	A	B	-	A	B	B
									C		C							C
64	umc185	B	A	-	A	B	A	B	B	B	-	A	A	B	B	A	A	B
65	bnl8.23	A	B	A	B	A	A	B	A	A	A	A	B	A	A	A	A	A
66	bnl7.50	A	A	A	A	-	-	B	A	-	A	-	A	B	-	-	A	A
						C	C			C		C			C	C		
67	php20024	A	A	A	A	-	-	B	B	A	A	A	A	B	A	-	B	A
						C	C									C		
68	umc124	A	A	-	H	-	A	-	-	B	A	A	A	B	B	A	B	B
69	umc84	A	A	A	H	B	A	B	B	-	-	A	B	B	B	H	A	B
70	npi286	B	A	B	A	A	A	B	B	B	B	A	A	A	B	A	A	B
71	umc82	A	B	B	B	A	A	A	A	B	B	A	A	B	B	A	B	A
72	umc82b	B	A	A	A	-	-	B	B	A	A	A	A	B	A	A	B	A
73	npi97	B	A	H	B	B	A	A	B	A	B	A	B	B	B	B	B	A
74	umc35	B	A	A	A	A	A	A	B	B	B	B	B	B	A	B	A	A
75	umc36	A	A	B	A	A	-	-	B	B	A	-	B	B	B	-	B	B
76	csu173	B	B	A	-	A	-	-	-	A	A	A	A	B	A	-	B	A
77	npi414	B	A	B	B	A	A	B	B	B	B	A	A	A	A	A	B	A
78	umc198	A	A	-	A	-	A	B	B	A	A	A	B	A	A	A	B	A
79	umc198b	B	B	-	H	-	A	A	A	A	A	-	B	A	-	A	A	A
												C			C			
80	npi400	B	A	A	H	A	A	A	B	A	A	B	A	A	A	A	A	A
81	umc81	A	A	B	A	A	B	B	A	A	A	B	A	B	B	B	B	B
82	umc117	B	B	B	A	A	B	B	-	-	-	A	A	A	A	A	A	B
83	umc113	A	A	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
84	bnl5.59	B	A	A	A	A	B	B	-	A	A	B	A	A	A	B	B	B
85	umc157	A	A	B	H	H	H	A	H	B	B	A	A	B	B	A	B	A

Appendix 3. Cont.

	RFLP Marker	101 G111	102 G112	103 G114	104 G115	105 G116	106 G117	107 G118	108 G119	109 G120	110 G121	111 G122	112 G124	113 G125	114 G126	115 G127	116 G128	117 G129
									RILs									
86	bnl5.09	A	A	-	A	-	A	A	A	A	A	A	A	A	A	A	A	A
					C		C			C		C		C	C	C		C
87	umc54	H	B	B	B	B	A	B	B	B	B	A	A	B	A	B	B	B
88	umc13	B	B	A	B	H	A	A	B	B	A	B	A	A	A	A	A	A
89	umc156	B	H	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A
90	umc38	B	B	B	B	B	B	A	B	A	A	B	B	A	A	B	B	B
91	umc190	A	A	A	A	A	A	B	A	A	A	A	A	B	A	A	A	A
92	csu86	A	B	B	B	B	B	B	A	B	B	A	B	B	A	A	A	A
93	bnl1.297	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	H	A
94	umc95	A	A	A	H	B	A	B	A	B	A	B	A	B	A	B	A	B
95	php20725	A	B	A	H	A	A	A	B	A	A	A	A	A	A	A	B	A
96	csu16	A	A	A	A	A	A	B	B	A	A	A	A	A	A	A	B	A
97	umc72	B	A	-	A	-	-	B	B	-	-	A	A	B	-	A	A	-
				C		C	C			C	C				C			C
98	umc43	A	B	-	B	-	A	B	B	-	A	A	A	-	-	A	B	-
				C	C	C				C				C	C			C
99	umc50	A	A	A	A	A	A	B	B	A	A	A	-	B	A	-	B	A
													C			C		
100	npi291	B	B	-	B	-	-	B	A	-	A	-	A	-	B	-	A	-
							C			C		C		C		C		C
101	npi291b	A	B	-	A	-	A	A	B	A	B	A	B	A	A	A	B	A
102	csu59	A	A	-	H	-	B	A	A	B	A	B	A	B	A	B	A	B
103	umc15	B	A	-	A	-	A	A	A	A	A	-	B	A	-	A	A	A
				C		C						C			C			
104	umc110	A	A	A	B	A	B	A	-	B	A	B	B	B	A	B	A	A
105	csu133	B	A	-	A	-	B	A	A	B	A	B	A	B	B	B	B	A
106	php20581	A	A	B	B	A	A	A	B	-	-	A	B	B	B	B	B	B
107	php20581b	A	A	A	A	B	A	A	B	-	-	B	A	A	B	A	A	A
108	npi105	A	A	-	-	A	-	B	A	-	-	-	-	A	A	B	B	A
				C	C		C					C	C					
109	npi287	B	A	-	H	-	A	A	A	H	A	A	B	B	B	B	A	B
110	php20508	H	B	-	A	-	A	A	B	A	A	A	A	A	B	B	B	A
111	UMC109	A	A	B	H	A	A	A	A	B	A	B	A	B	B	A	A	B
112	bnl16.06	B	A	A	A	A	A	A	B	-	-	-	B	-	A	-	A	A
										C	C	C		C		C		
113	bnl16.06b	B	B	A	B	B	B	B	A	B	B	B	B	B	B	A	B	B
114	umc136	A	B	B	B	A	B	A	B	-	-	B	B	B	B	A	A	A
115	bnl14.07	A	A	A	H	A	A	A	H	B	B	B	B	B	A	B	A	A
116	csu50	B	B	A	A	-	A	A	B	A	A	A	A	A	A	A	A	A
117	php20075	B	B	A	H	A	A	A	B	A	A	A	A	A	A	A	B	A
118	umc132	B	B	A	A	A	A	A	A	A	A	A	B	B	A	A	B	A
119	umc19	A	B	A	H	A	A	A	A	A	A	A	A	A	A	A	A	A
120	npi393	B	A	A	A	B	-	B	-	A	A	A	B	-	B	A	B	A
121	umc123	B	A	A	A	B	B	A	-	A	B	A	A	A	B	A	B	B
122	csu30	A	B	A	A	A	-	B	-	A	A	A	A	A	B	B	B	A
123	npi239	A	A	A	A	A	A	A	B	-	-	A	A	A	-	-	B	A
										C	C				C	C		
124	umc103	B	A	-	A	-	A	B	B	A	A	A	B	A	A	A	A	A
125	umc164	B	A	A	A	B	A	A	A	A	B	B	B	B	B	B	B	A
126	npi321	B	B	B	B	B	A	B	B	A	B	B	A	B	B	B	B	A
127	npi114	A	A	A	A	A	A	A	B	A	A	A	A	B	A	B	B	A

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