3

QTL Analysis of Multigenic Disease Resistance in Plant Breeding

James D. Kelly and Veronica Vallejo

3.1 Introduction

Multigenic or quantitative disease resistance has challenged plant breeders working to develop disease resistant crop cultivars. The challenge to incorporate into new cultivars equivalent levels of resistance that existed in the original genetic resistance stock(s) is formidable, given the apparent complexity of quantitative resistance. Environmental factors, complex multigenic inheritance, plant avoidance, and escape mechanisms combine to hamper the efforts of breeders working to incorporate multigenic resistance into future cultivars. Breeding for quantitative resistance is more formidable than for qualitative resistance traits as more complex and lengthy breeding procedures are needed to effectively incorporate adequate levels of quantitative resistance into new crop cultivars. The expression of quantitative resistance in many instances is partial, not absolute, and the control of resistance appears to be governed by many genes acting cumulatively. The rating of genotypes for disease development in field or greenhouse becomes more subjective due to interactions with environmental and plant morphological factors, requiring additional testing and replications to validate their accuracy. In the literature, many nonspecific and complex resistance mechanisms associated with quantitative resistance have been grouped under the broad general headings of horizontal resistance, polygenic resistance, partial resistance, or durable resistance, which suggests complexity but contributes little to resistance breeding. Current analytical molecular tools, however, are making the breeding of quantitative resistance more effective and new insights on the magnitude and location of such resistance loci may assist plant breeders in better exploiting this type of resistance in future crop cultivars. Quantitative Trait Loci (QTL) analysis is a valuable tool for genome exploration and the investigation of multigenic traits. The focus of this chapter is to review the body of work devoted to the identification of QTL controlling quantitative disease resistance in crops and the exciting implications of the implementation of QTL analysis to dramatically enhance disease resistance breeding. QTL analysis is rapidly changing the way scientists view disease resistance and the time-held concepts and importance of major and minor gene resistance. In

order to discuss the implications of QTL analyses in resistance breeding, we first attempt to bring some clarity to the terminology and controversial theories that have historically competed for recognition in the breeding literature.

3.2 Terminology

3.2.1 Complex Multigenic/Quantitative Traits and Durable Resistance

The terms multigenic and quantitative are somewhat interchangeable but multigenic implies knowledge of gene action, hence genotype, whereas quantitative implies characterization based on observation, hence phenotype. Not all quantitative traits are multigenic in terms of gene action as environmental factors combine to influence phenotypic expression of complex traits. As authors, we favor the use of the term quantitative; in most instances, breeders base decisions on phenotype, since gene action of complex traits is not always known. Most, but not all, complex resistance traits are controlled by multiple loci. A complex trait is one that does not fit simple Mendelian ratios (Young, 1996). Resistance phenotypes that do not fit discrete categories and are measured quantitatively are assumed to be controlled by multiple loci referred to as QTL. QTL for resistance refer to locations on the genome that are involved in quantitative resistance, but are not informative of the function (Lindhout, 2002). Quantitative resistance has been assumed to be more durable than resistance conferred by a single dominant gene (Parlevliet, 2002). Durable resistance is resistance that remains effective during prolonged and widespread use in environments favorable for the spread of the pathogen (Johnson, 1984). This definition does not imply gene action or resistance mechanism. It is generally assumed, however, that for resistance to be durable it must be under polygenic control. This term implies the role of many genes with the implication that each "gene" has a small but cumulative effect on the expression of resistance in the host. The explanation is based on the inability of scientists to identify clearly major gene effects controlling resistance. Causes for the inability to identify major gene effects are based on (1) absence of major genes and role of minor genes in resistance expression, (2) large environmental effects on major or minor genes which result in non discrete resistance categories, (3) mixtures of pathogenic races that obscure major gene effects, (4) pathogen interactions, (5) interaction with plant morphological avoidance mechanisms or disease escape due to difference in phenology between genotypes, and (6) possible confusion with tolerance mechanisms where specific genotypes tolerate higher levels of disease infection without a corresponding reduction in productivity.

Durability of resistance is viewed as a quantitative trait as it can range from ephemeral to highly durable (Parlevliet, 2002). Despite the clear recognition that ephemeral resistance is characterized by major gene resistance to those pathogens known as specialists (Lamb et al., 1989), the nature of durable resistance is less clear. Durable resistance can be oligogenic particularly against viral pathogens

(Harrison, 2002), but more commonly resistance is quantitative and durable to those pathogens known as generalists that pathogenize a wide host range. The long held theory that polygenic resistance is more durable (van der Plank, 1968) is now being refuted due to the ability of certain pathogens (*Mycosphaerella graminicola*) to overcome both qualitative and partial resistance in wheat (*Triticum aestivum*; Mundt et al., 2002) and reports that monogenic resistance can be durable (Eenink, 1976). For example, the genetic control of bean common mosaic virus (BCMV) conditioned by the dominant *I* gene (Ali, 1950) has been effective in common bean (*Phaseolus vulgaris*) for over 40 years. No reports exist of breakdown of the *I* gene resistance to new evolving strains of BCMV, despite the extensive deployment of the *I* gene in bean cultivars worldwide.

3.2.2 Horizontal and Vertical Resistance

Since its introduction by van der Plank (1968), the concept of vertical and horizontal disease resistance has been an invaluable hypothesis for plant breeders needing to conceptualize the nature of the disease resistance in a specific crop/pathogen interaction. The need to understand the interaction is essential to formulate a strategy for resistance breeding based on the type of resistance (qualitative or quantitative) present in the host, and the nature and type of variability in the pathogen. Breeders rarely choose the type of resistance with which they work, as factors outside their control influence that decision. Such factors include: the nature of the pathogen (specialist or generalist), host range (wide or species specific), type and availability of resistance mechanisms present in the host (gene-for-gene vertical resistance, nonspecific avoidance), the level of resistance (complete or partial) needed in the crop, and the difficulty of distinguishing partial resistance in the presence of major resistance genes.

van der Plank (1968) defined vertical resistance as race-specific and horizontal resistance as race-nonspecific. The terminology used to describe these types of resistance can be confusing as it includes both the genetic control of resistance and observations on the performance of resistance in the field. The term "quantitative resistance" has often been synonymous with "horizontal resistance", implying, by van der Plank's definition, that quantitative resistance is race-nonspecific. QTL for resistance can be identified using specific races of the pathogen that behave as specialists, but the more common instance is the association of QTL with resistance to a pathogen that is a generalist in its mode of action. Certain QTL are related to strain-specific resistance whereas others are strain-nonspecific (Young, 1996). Qi et al. (1998) mapped QTL for resistance to leaf rust (*Puccinia hordei*) in barley (*Hordeum vulgare*) and identified several QTL (*Rphq1-6*) linked to resistance using a single isolate of *P. hordei*. A subsequent study using another isolate (24) (Qi et al., 1999) found that four other QTL (*Rphq7-10*) were specific for isolate 24 and two QTL (*Rphq5* and *6*) were specific to a different isolate of *P. hordei*. Isolate-specific QTL for resistance have also been found for bacterial wilt (*Pseudomonas solanacearum*) in tomato (*Lycopersicon esculentum*; Danesh and Young, 1994) and late blight (*Phytophthora infestans*) in potato (*Solanum tuberosum*;

Leonards-Schippers et al., 1994). These studies lend support to the "minor-gene-for-gene" hypothesis that there exist small but significant cultivar/isolate interactions (Parlevliet and Zadoks, 1977) that appear qualitative on an individual basis but behave cumulatively in a quantitative manner.

The discovery that QTL for resistance can be race-specific opens the possibility that these QTL are involved in similar resistance mechanisms as major race-specific *R*-genes. In the concept of race-specificity of major *R*-genes, elicitor molecules encoded by an *Avr* gene in the pathogen are perceived by the plant cell by binding of this ligand to a receptor encoded by the *R*-gene. Binding of this ligand by the receptor triggers a signal transduction pathway leading to the hypersensitive response (HR), which is characterized as accelerated, localized, plant cell death, and an incompatible reaction (Hammond-Kosack and Jones, 1997). Vleeshouwers et al. (2000) studied the interactions between *P. infestans* and *Solanum* spp. by examining the differential reactions of a diverse series of wild species. They found that in partially resistant species, HR was induced between 16 and 46 hours, and had variable lesions of five or more dead cells from which, in some cases, hyphae were able to escape and establish disease. These results, and other studies discussed in Sections 3.8 and 3.9 of this chapter, indicate that the HR of the partially resistant *Solanum* species used was quantitative in nature. Partial resistance refers to quantitative resistance not based on HR (Parlevliet, 1975); thus, partial resistance should not be used synonymously with QTL unless the type of gene action is known.

The types of mechanisms functional in horizontal resistance are commonly referred to in the literature as multigenic or polygenic. A more appropriate terminology that would benefit breeders in distinguishing the types of host resistance is based on the classification of the trait as either qualitative or quantitative resistance. Breeders are familiar with these types of traits and can formulate effective breeding schemes to incorporate such traits into new cultivars. When treated as quantitative resistance, the breeder has a body of information on the expression of these types of traits and methodologies to effectively manipulate such traits (Hallauer and Miranda, 1981). The basis for quantitative inheritance is as complex as the traits being studied since the range of traits under quantitative control in most crops plants dramatically out-number those under qualitative control. Progress in the improvement of quantitative traits has lagged behind similar efforts to improve simply inherited traits due to their complexity, lack of complete expression, inconsistent screening methods, and the need for widespread multilocation testing. The lack of progress is best understood when differences in inheritance patterns between qualitative and quantitative resistance are compared.

The relative contribution and stability of the QTL to disease resistance is another important criterion of QTL analysis. Quantitative genetic theory implies that many minor genes control quantitative traits, but what is not known, is the differential effect of different minor genes. In the case of disease resistance, QTL analysis reveals that resistance may be controlled by a few QTL with major effect (high coefficient of determination, $R^2 > 35\%$), and a number of QTL with relatively minor effects ($R^2 < 15\%$) (Kolb et al., 2001; Young, 1996). For example, one QTL

conditioning resistance to downy mildew (*Sclerospora graminicola*) in pearl millet (*Pennisetum glaucum*) accounted for 60% of the phenotypic variation whereas another accounted for only 16% of the variation associated with resistance (Jones et al., 2002). Clearly such information provides breeders with a clear choice on which QTL to emphasize in breeding for resistance, along with the tools to achieve that objective.Other factors that influence the effectiveness of QTL analysis are the potential interaction between QTL, and their stability across environments and populations, and possible linkages with other traits. Generally breeders shy away from population and environmentally sensitive QTL as they are too restrictive to the overall goals of most breeding programs (Asins, 2002).

3.3 Historical Perspective

Due to the complex nature of inheritance, classical Mendelian techniques were not applicable to quantitative traits, and in the early part of the 20th century, quantitative genetics emerged as a specialized branch of genetics to address issues related to traits under quantitative genetic control. Until recently, quantitative genetics relied on biometrical approaches that deal mainly with the characterization of multiple factors affecting a quantitative trait and partition the phenotypic variance into its genotypic and environment components (Hallauer and Miranda, 1981; Sprague, 1966). From these statistical procedures, several parameters could be estimated including the approximate number of loci influencing the trait, gene action, gain from selection estimates, and the degree to which the loci interacted with other loci and the environment to produce the observed phenotype. These approaches, however, were limited in the sense that they were not able to characterize any *one* specific locus that contributed to the trait, either its location or size of effect. The biometrical information did provide breeders with information on type of gene action that suggested the most appropriate breeding methods to use to optimize or fix favorable gene action controlling the quantitative trait. Many of the mating procedures, however, were limited to specific crops such as maize (*Zea mays*) due to the pollination mechanisms and reproductive biology of the crop.

Sax (1923), accredited with being the first to describe the theory of mapping quantitative traits, showed that loci involved in a quantitative trait (seed size in common bean) were associated with a qualitative trait (seed-coat pigmentation). Another pioneer in the characterization of quantitative traits, Thoday (1961), suggested the need to exploit the association with qualitative traits as a means to locate the polygenes involved in the control of a complex trait. He astutely noted, however, that the limiting factor in using this strategy was the availability of suitable markers. With the advent of molecular markers that are sufficiently numerous to provide adequate genome coverage, this is no longer a limitation and therefore, QTL mapping, at least in theory, can resolve any additive gene of small effect as Mendelian through associations with a marker locus. The era of molecular markers commenced with the discovery of isozyme techniques (Hunter, 1957; Smithies, 1955) and quickly progressed to DNA-based marker systems, first of which were

RFLP (Botstein et al., 1980) followed by PCR-based molecular markers (RAPD, SCAR, SSR, AFLP; Michelmore et al., 1991; Vos et al., 1995; Weber and May, 1989; Williams et al., 1990). For a more complete review of the different marker systems available for mapping, see Staub et al. (1996).

The basic concept of QTL mapping is very simple: to find significant associations between marker genotypes and quantitative phenotypes in a large controlled, experimental cross between two parental genotypes. A conceptual diagram of QTL mapping is provided by Young (1996). In practice, however, there are many issues: (1) population size, (2) parental selection, (3) population type, (4) marker efficiency, (5) phenotypic data that breeders need to consider, (6) map density, and (7) data analysis that influence QTL analysis.

3.4 Mapping Considerations

3.4.1 Population Size

The purpose of a mapping population, in essence, is to simplify partitioning of genetic variance components to provide a clear genetic interpretation and genomic data analysis. The mating design of a mapping population is important for making the relationships among the polymorphic markers and traits of interest detectable and tractable. The effective population size for QTL analysis is a very important consideration that has a direct impact on the resolution of the map and the accuracy of the QTL location. Population size also affects the genetic gains breeders achieve using marker-assisted selection (MAS). If the population is not large enough ($<$100 individuals) in a QTL analysis, certain putative QTL will not be detected and therefore gains using these candidate QTL in MAS will be reduced. Large population sizes ($>$200 individuals) are not always feasible due to the space and time constraints on the researcher, therefore, some strategies have been implemented to maximize information from smaller populations, including selective genotyping (Lander and Botstein, 1989) and DNA pooling of similar phenotypes (Michelmore et al., 1991).

In QTL analyses, selective genotyping and bulked segregant analysis (BSA) (Michelmore et al., 1991) have been utilized to efficiently screen large numbers of polymorphic markers, without having to genotype entire segregating populations. Selective genotyping involves the identification of a subset, usually 10–14% of the genotypes that possess extreme phenotypes of the population. By this method, breeders can obtain equal or greater information about QTL than from mapping of randomly chosen individuals. A small percentage of the total genotypes that exhibit extreme phenotypic values for the trait of interest can be grouped (bulked) together, and either analyzed as individuals, or through BSA, where the DNA of the similar phenotypes are pooled. BSA is most often used when mapping genes with major effect. BSA may have limited application to QTL analysis due to factors such as dominance and non-Mendelian segregation that decrease the effectiveness. Selective genotyping and BSA has been used successfully in the identification of QTL for quantitatively-inherited traits related to disease resistance (Chen et al., 1994; Miklas et al., 1996; Schneider et al., 2001). Another application of BSA in

QTL analysis is in fine mapping of a QTL position. To find additional markers linked to a particular genomic region, pools are created based on alternate alleles at a marker locus, providing a very efficient method for screening large numbers of markers to saturate a QTL region (Giovannoni et al., 1991). Paterson (1998) states that rare QTL with large effects can be fixed in the phenotypically extreme individuals, and therefore may be detected as a chromosome segment polymorphic between contrasting DNA pools. Most QTL with smaller effects, however, will remain heterogeneous in the DNA pools and will not be detected. To detect many QTL with smaller effects, Paterson suggests a comprehensive mapping approach. Despite the view that DNA pooling might be useful in the identification of QTL of very large effect but unlikely to permit the comprehensive identification of the majority of QTL affecting a complex trait (Wang and Paterson, 1994), breeders have successfully used BSA in the identification of QTL for disease resistance (Miklas et al., 1996; Young, 1996).

3.4.2 Selection of Parents

When the objective of the research is to search for genes controlling a particular disease resistance trait, adequate genetic variation for resistance must exist between the parents. There must be sufficient variation between the parents at the DNA sequence level and at the phenotypic level for the trait of interest. The choice of parents may be restricted by the availability of resistance but breeders usually make a decision as to the level of diversity of the parents of the mapping population. Wider diversity between parents may be desirable to allow the mapping of traits in addition to the targeted resistance source, or breeders may need to work with genetically similar parents to avoid the interaction of other traits such as plant morphology and phenology on the expression of resistance in the field (Lindhout, 2002).

3.4.3 Population Type

The most commonly used mating types in QTL analyses are F_2 and backcross (BC) populations. The disadvantage of these types of populations is that they are unique and progeny cannot be propagated, so breeders are unable to recreate the same population for further testing. Recombinant inbred lines (RIL) and double haploid (DH) homozygous lines can be used to avoid this problem because the lines are maintained by selfing, allowing marker-trait associations to be scored across multiple environments in a completely homozygous background. RIL are developed initially by self-pollinating the F_2 generation for up to10 generations using the single-seed descent method (Burr and Burr, 1991). DH lines are produced by the induction of diploid gametes by tissue culture. In this case, haploid gametes from F_1 parents are chemically treated to induce the doubling of the chromosome number (Jensen, 1989; Knapp, 1991; Knapp et al., 1990). The technology to generate DH lines, however, is not available in all crops. Although RIL populations take longer to generate, they have become the cornerstone of many QTL analyses as they can be easily duplicated for widespread testing. The utility of phenotypic-based DNA pools on the isolation of QTL in different genetic populations was

assessed by Wang and Paterson (1994). The effects of population size, portion of population selected, magnitude of phenotypic effects of individual QTL alleles (QTL allele effects) and effects of both dominance and deviations from Mendelian segregation ratios were considered. Backcross populations were better than F_2 populations, but were less efficient than RIL or DH populations in detecting QTL. To detect QTL using phenotypic-based DNA pools, Wang and Paterson (1994) suggested using wide crosses, large homozygous populations such as RIL and DH populations where the replication of phenotypic data is easily facilitated by the use of homozygous populations.

3.4.4 Marker Efficiencies

The choice of markers is dependent on those available in each crop, but PCR markers are the clear choice over RFLP markers because of cost and convenience. Many of the major crops such as soybean (*Glycine max*) have numerous microsatellite or SSR markers (Cregan et al., 1999a) and/or SNP and CAPS markers available for mapping. In minor crops where sequence-based markers are not yet available, breeders may utilize AFLP markers or even RAPD markers. Different marker systems have varying levels of resolution to detect genome variations. Codominant markers are generally preferred over dominant markers in certain populations. Dominant marker types are not recommended for F_2 populations because in repulsion linkage phase the dominant markers provide low information content on linkage (Paterson, 1998). This disadvantage is less acute when mapping more homozygous RIL populations. In a BC population, if the recurrent parent is recessive for the dominance loci, dominant and codominant markers are equivalent in terms of genomic analysis.

3.4.5 Phenotypic Data

Limitations of QTL analyses rarely lie in the lack or inability to find useful polymorphic markers associated with disease resistance, but reside in the accuracy of trait analysis. The collection of the phenotypic data used to conduct the analysis is challenging in terms of the establishment of rating scales for disease evaluation, actual evaluations and data collection, seasonal and location effects of the environment and the structure and size of the genetic population being evaluated. All of these factors can contribute unexplained variability to the data set and need to be considered by the researcher conducting the analysis. In the vast majority of cases the weakness of the QTL analysis resides in the phenotypic data used to conduct the analysis and less in the density of markers available for mapping. The most common rationale in mapping disease resistance traits is to generate a segregating population where individuals exhibiting the extreme expression(s) of the resistance trait can be identified for mapping purposes. In the case of the oligogenic traits, such contrasting individuals can easily be identified in early generations such as the F_2, whereas in mapping of quantitative resistance, individuals can only be identified on a progeny basis in later, more homozygous, generations. Since the

expression of quantitative resistance can be effected by environmental conditions, the resistance trait needs to be measured over locations and/or years. The need to create, replicate and evaluate more homozygous lines results in significant delays in all QTL analyses of quantitative resistance. All sound QTL analyses must be based on clear reproducible quantitative phenotypic data generated from the genetic population segregating for the resistance trait. Breeders need to be aware that many QTL analyses fail to identify true or significant effects simply due to weak or questionable phenotypic data collected on the disease resistance trait. Marker-assisted selection must be based on a data set that is uncompromised in quality and reproducibility.

3.4.6 Map Density

QTL discovery may be conducted with or without using an existing genetic linkage map. Not all crop species, such as the octoploid strawberry (*Fragaria* x *ananassa*), have a well-saturated linkage map with even distribution of markers across the genome. In such instances, QTL discovery is accomplished by simply finding a statistically significant association between a phenotype and a marker. The marker is often detected by screening random primers against a population segregating for the quantitative trait. Although this approach may appear inefficient, valuable QTL for resistance to root rot (*Fusarium solani* f. sp. *phaseoli*) have been discovered in common bean by this method (Schneider et al., 2001). In crop species such as soybean that do have linkage maps with even distribution of markers across the genome (Cregan et al., 1999b), marker density can have an impact on the accuracy or resolution of the QTL location. In general, markers should be evenly distributed with at least one marker every 5 cm. Genome coverage and map density can be influenced by a number of different factors: size of the genome, population size and type, mapping strategy used, distribution of crossovers in the genome, and number of markers (Liu, 1998).

3.4.7 Data Analysis

Three main methods of data analysis are generally used in evaluating linkage between markers and a phenotype. These methods include: single-marker analysis, interval mapping, and composite interval mapping.

Single-Marker Analysis

In single-marker analysis, the trait value distribution is examined separately for each marker locus. This can be done using a simple t-test, analysis of variance, linear regression, or likelihood ratio test and maximum likelihood estimation. Due to the simplicity of this analysis, SAS (Statistical Analysis Software, SAS Institute, Cary, NC) can be used. There are a few disadvantages of this type of analysis. One disadvantage is that the QTL location and the putative QTL genotypic means are confounded, which reduces the statistical power of this analysis. This

is a particularly important consideration when working with a low-density map. Another disadvantage to single-marker analysis is that the QTL cannot be precisely mapped due to the non-independence among the hypothesis tests for linked markers that confound QTL effect and position (Liu, 1998). This method is therefore more suited to a study where the goal is to simply detect QTL linked to a marker rather than to accurately map and estimate their effects.

Interval Mapping

The limitations of single-marker analysis prompted Lander and Botstein (1986a; 1986b; 1989) to propose an interval mapping (IM) method to position QTL. In IM, a separate analysis is performed for each pair of marker loci using one of the three approaches: likelihood (Lander and Botstein, 1989), regression (Knapp et al., 1990), and/or a combination of both methods. The IM method provides increased power of detection of QTL and more accurate QTL positioning when compared to single-marker analysis (Liu, 1998). The disadvantages of this method are that the number of QTL cannot be resolved, the exact position of the QTL cannot be determined, and the statistical power, although higher than single-marker analysis, is still relatively low. These problems can result from linkage or interactions between QTL, and limited information in the model (Liu, 1998). The outcome of this method is highly influenced by background QTL that result in low wide peaks which mask the appearance and positioning of multiple linked QTL.

Composite Interval Mapping

Composite interval mapping (CIM) is a combination of interval mapping and multiple linear regression (Zeng, 1993, 1994). This method considers a marker interval and a few chosen markers in each analysis. These chosen markers are used to reduce background effects of other linked QTL in the analysis of a marker interval. The result of CIM is to define the most likely position of the QTL with more precision and greatly increase the resolution of the analysis, which is the most important advantage of CIM over single-marker analysis and IM. Since there are more variables in the model, CIM is more informative and efficient, and results can be presented using the log likelihood ratio test statistic plot and the LOD score plot for all possible genome positions.

3.5 Applications of QTL Analysis to Disease Resistance Breeding

QTL analysis has enhanced our understanding of quantitative resistance in a number of key areas by revealing the location and size of loci controlling disease resistance. Locating resistance loci has confirmed the interaction between resistance traits that control physiological processes and those traits influencing plant morphology and phenology that control disease avoidance and/or escape in a field

setting. By locating loci for quantitative disease resistance on different linkage groups, QTL analyses provide unique opportunities to pyramid resistance loci in order to restore higher levels of resistance lost in many cases after crossing with a highly resistant source (Vertifolia effect; van der Plank, 1968). While the practical application of MAS for quantitative traits has yet to be fully realized in breeding, many studies recognize its potential to facilitate improved disease resistance controlled by quantitative traits (Asins, 2002; Faris et al., 1999; Jones et al., 2002; Kolb et al., 2001; Lindhout, 2002; Lubberstedt et al., 1998; Mangin et al., 1999; Miklas et al., 1998; Pilet et al., 1998; Schechert et al., 1999). QTL-marker associations may also provide a basis for a greater understanding of quantitative disease resistance through the identification of loci that influence resistance to more than one disease (Ariyarathne et al., 1999). The application of MAS in breeding for quantitative resistance should have the most impact in breeding for resistance to soilborne pathogens such as *Fusarium* and *Sclerotinia*. Screening for resistance in the field is both destructive and complicated by the interaction of other soil borne pathogens (root rot complex), seasonal environmental factors, and plant morphological traits that contribute to disease avoidance or escape which hinders the normal selection procedures (Tanksley et al., 1989). Replacing laborious screening of quantitatively inherited traits with MAS has several advantages in a breeding program. Breeding for quantitative resistance can be enhanced with the discovery of QTL for resistance that would allow for the indirect selection of resistance without confounding effects of environmental factors. In the absence of candidate QTL, breeders were often forced to cross "blindly" in the hope that they were combining resistance sources (loci) but with the discovery of QTL, breeders can target specific loci on different linkage groups and combine these in future resistant cultivars. The breeding literature has many examples of attempts to transfer quantitative resistance to potential new cultivars that have resulted in the transfer of partial levels of resistance. When breeders lack the tools to identify putative QTL involved in resistance they are equally ineffective in transferring all the resistance QTL to future cultivars.

3.6 Use of Multitrait Bulking Methods in QTL Analysis

Disease development can be influenced by plant morphological and phenological factors that must be considered by breeders working with quantitative disease resistance. For example, a number of agronomic traits, including growth habit, canopy height and width, branching pattern, lodging, days to flower and maturity have been shown to be significantly associated with white mold (*Sclerotinia sclerotiorum*) development in common bean (Kolkman and Kelly, 2002). The interaction of such traits on the expression of the disease resistance trait complicates breeding for resistance. Morphological traits such as plant architecture afford disease avoidance, whereas phenological traits such as early flowering afford disease escapes in many instances (Coyne, 1980; Kolkman and Kelly, 2002). Since both types of traits influence disease reactions in the field, both need to be considered in

a QTL analysis of specific disease resistance traits. In the selective genotyping of quantitative resistance, the identification of individuals with extreme expression of disease resistance may result in the selection of individuals that exhibit undesirable morphological and/or phenological traits due to interaction of these traits on the expression of disease resistance. Highly resistant individuals may result not from the expression of true physiological resistance but from a combination of such agronomically undesirable traits as short plant stature, or extremely early flowering or maturity that result in individuals with no agronomic or yield potential for commercial production. Such individuals serve no potential as parents, or cultivars as their agronomic weaknesses outweigh their low disease resistance ratings. This problem becomes particularly acute in QTL analyses where selective genotyping is used to assist the breeder in identifying the extreme expression(s) of disease resistance, but results in an analysis of the extreme expression of agronomic traits that escape or avoid the disease, resulting in the mapping of traits associated with agronomically inferior individuals.

In the mapping of QTL associated with white mold resistance in common bean, DNA bulks comprised solely of a small number of lines in the extreme phenotypes may not adequately represent useful resistant genotypes in the population. Since the use of selective genotyping for a trait as complex as resistance to white mold may be hindered by the limitation of a set of DNA bulks based on disease reaction alone, Kolkman and Kelly (2003) compared the efficiency of single and multitrait bulking strategy for the identification of QTL associated with white mold resistance. The multitrait bulking strategy utilized multiple traits (MT) to develop contrasting DNA bulks for use in genotyping as opposed to traditional single trait bulks. The traits selected in the MT bulks included disease reaction and also flowering range and yield to avoid the indirect selection of resistant, low-yielding genotypes with inferior agronomic traits such as very early or late flowering that would effect local adaptation (Kolkman and Kelly, 2003). The results of the study indicated that both single- and multi- trait bulking strategies identified QTL for resistance to white mold on one linkage group. However, eight molecular markers on a second linkage group B7 were identified using the MT bulks, whereas the single-trait bulk for disease incidence alone would not have identified the most closely linked markers to the QTL conferring resistance to white mold on B7. The disease ratings in the selected individuals within the resistant MT bulks were higher than those of the single-trait disease resistant bulk, suggesting that the disease resistant bulk may have included genotypes with greater avoidance mechanisms that significantly reduced yield but were potentially commercially unproductive. The authors concluded that genotyping a chosen set of individuals with specific phenotypes, based on a priori knowledge of the traits that are segregating in the population that may affect the desired phenotype, was an efficient method to detect markers linked to the resistance phenotype which would not have been detected in the single-trait disease resistant bulks alone (Kolkman and Kelly, 2003).

In soybean, two of the three QTL associated with disease resistance to *S. sclerotiorum*, were also associated with plant avoidance mechanisms, such as plant

height, lodging, and date of flowering (Kim and Diers, 2000). The authors speculate that the third QTL, which was not significantly associated with escape mechanisms, may be involved in physiological resistance to *S. sclerotiorum*. Plant avoidance mechanisms may also play an important role in resistance to *S. sclerotiorum* in sunflower (*Helianthus annuus*). QTL accounted for up to 60% of the leaf resistance and up to 38% of the capitulum resistance in sunflower. Apical branching pattern was suggested as exhibiting the best resistance to infection of the capitulum (Mestries et al., 1998), whereas the association between days to flowering and resistance to *S. sclerotiorum* in sunflower was dependent upon the population (Castano et al., 1993). Clearly, MAS allows for the identification and selection of superior genotypes without having to employ undue effort in phenotyping large number of individuals. The difficulty in detection of desirable phenotypes, due to factors such as environmental variation, hinders normal selection procedures for important quantitative traits, and increases the importance of MAS. DNA bulks comprised solely of a small number of lines in the extreme phenotypes may not adequately represent resistant genotypes in the population. DNA pooling strategies based on a priori knowledge about the population should help resolve useful markers linked to QTL, and discern the location of QTL regions (Wang and Paterson, 1994). Genotyping multiple traits that are related to the trait of interest have been effective in identifying QTL that may not be detected through screening extreme phenotypes (Kolkman and Kelly, 2003; Ronin et al., 1998).

3.7 Identification of Novel Disease Resistance Sources Using QTL Analysis

Interspecific hybridization has been used to improve disease resistance in many crop species (Hadley and Openshaw, 1980). The inheritance of the resistance is not always known as breeders rarely conduct genetic studies in the alien species but focus on the successful transfer of the resistance to the cultivated species. As a result the assumption is often made that resistance in the alien species is novel and worth the substantial efforts needed to transfer resistance. Mapping of QTL has provided new information on resistance sources integrated from other species. Lack of adequate levels of resistance to common bacterial blight (CBB; *Xanthomonas axonopodis* pv. *phaseoli*) in common bean has forced bean breeders to find resistance in the related tepary bean (*P. acutifolius*) species. Impetus to use interspecific crosses came from early work by Honma (1956) who reported a successful interspecific hybrid between common and tepary bean that has become the focus of CBB resistance breeding for the last 40 years. Progress in breeding for resistance to CBB in common bean has been modest as resistance is quantitative, largely influenced by environment and pathotype, and functional in different organs, leaf, seed, or pod depending on resistance source(s). The complexity of resistance to CBB where different QTL conditioned resistance in young and adult tissues to different strains of the pathogen, or where one genomic region possessed a factor(s) which influenced resistance in all three tissues, seeds, leaves, and pods,

while another QTL only influenced resistance within a single plant organ has been demonstrated (Jung et al., 1999; Kelly et al., 2003). Such complexity in disease expression has limited progress in breeding for resistance to CBB.

Despite these difficulties, QTL analyses of resistance to CBB in common bean has resulted in the identification of four major QTL associated with resistance on four different linkage groups that provides breeders with the possibility of combining QTL to enhance resistance. One of the most revealing findings provided by QTL analyses concerns the resistance source originally believed to have been derived from the tepary bean (Honma, 1956). This source has proven to be of common bean origin, not tepary as previously thought (Miklas et al., 2003). The QTL for resistance on linkage group B10 is found only in common bean germplasm and is absent from all tepary bean resistance sources tested (Miklas et al., 2003). The resistance QTL on linkage group B10 co-segregated with resistance in common bean progeny tested for reaction to CBB confirming that resistance was not derived from tepary bean in the original cross. QTL mapping, therefore, provides an opportunity to verify the uniqueness of resistance sources prior to using them directly in breeding programs.

Another advantage of QTL analysis is the identification of previously unknown resistance sources. QTL have revealed that different genetic sources present in related species may not always represent new or novel resistance loci. These exotic sources may be assumed to be unique, as the resistance sources are not characterized if present in a related species. Genetic studies are not routinely conducted on alien or exotic species to determine their relationship, so a savings in time and resources results from knowing if an exotic resistance source does or does not carry a unique QTL. Given the lack of adequate resistance sources to CBB in common bean, resistance has been successfully introgressed from different tepary accessions into common bean (McElroy, 1985; Scott and Michaels, 1992). QTL analyses of these resistance sources for CBB derived from different interspecific tepary bean sources mapped to linkage groups B6 and B8 on the common bean map (Miklas et al., 2000). These resistance sources are clearly derived from tepary bean, as the QTL are absent in susceptible common bean genotypes and present in resistant tepary bean germplasm. One of these sources with QTL on B6 and B8 is XAN 159 (McElroy, 1985), the most widely deployed source of resistance currently used by bean breeders. A second tepary derived resistance source OAC 88-1 was developed independently (Scott and Michaels, 1992). Since no genetic studies were conducted on the tepary bean sources, the assumption that different resistance sources had been successfully introgressed into common bean persisted. This assumption has proved false as the QTL from XAN 159 known as SU91 mapped to the same location on B8 as the R7313, the QTL from OAC 88-1 (Miklas et al., 2000). This represents a duplication of effort and resources, given the difficulty of making interspecific crosses between tepary and common bean and the need to employ embryo rescue in the procedure. Apparently, the same source of resistance was independently introgressed into common bean without prior knowledge of the genetic similarities of the tepary bean accessions. QTL analyses can serve a vital role in distinguishing resistance sources based on their location in the genome.

Those QTL that map to the same location most likely condition similar resistance; with that knowledge, intelligent decisions can be made on the choice of sources to introgress when time consuming interspecific crosses are required. Finally, the potential to pyramid four QTL, two from common bean and two from tepary bean, into a single bean genotype opens up the exciting possibility of developing common bean cultivars with CBB resistance levels (Singh and Munoz, 1999) equivalent to those in the original tepary bean sources.

3.8 Colocalization of QTL with Resistance Genes

The focus of QTL analysis has changed recently from simply discovery of QTL associated with quantitative disease resistance to determining the biological function underlying the QTL. Knowledge about the biological functions of QTL will help breeders develop cultivars with more durable resistance as well as elucidate the mechanisms behind quantitative resistance. Understanding the function of genes that confer quantitative resistance will provide breeders with mechanistic information that can be used to make more informed and prudent decisions as to why QTL for resistance may be more durable. The term QTL is not very descriptive, only referring to a specific genomic location involved in the quantitative disease resistance, and does not provide information about the function of those genes. By studying the function of other genes that map to the same genomic regions as QTL, information on the mechanisms influencing resistance conferred by QTL may be elucidated. The role that some QTL play in resistance through their association with the HR (Vleeshouwers et al., 2000) may provide information on the biological function of QTL in the resistance reaction.

3.8.1 QTL that Colocalize with Major Genes for Resistance

QTL may be Allelic Variants of Qualitative Resistance Genes

There are two broad categories of genes involved in plant defense response: *R*-genes (those involved in the recognition of the pathogen), and defense response (DR) genes (those involved in the general defense response of the plant). One instance of colocalization is the mapping of a QTL to the same genomic regions where previously mapped *R*-genes reside. The existence of quantitative and qualitative resistance genes in the same genomic regions favors the consideration that QTL, which confer intermediate resistance, may correspond to allelic versions of qualitative resistance genes. This is consistent with the hypothesis that mutant alleles of qualitative genes that affect quantitative traits are one extreme in the spectrum of alleles (Robertson, 1989). A possible explanation at the molecular level is that qualitative mutants may result from loss of function mutations whereas quantitative alleles may result from mutations that produce a less efficient gene product resulting in differences in phenotypes. Support for this theory comes from a study on rice (*Oryza sativa*) using 20 RFLP marker loci associated with

quantitative resistance to rice blast *Pyricularia oryzae* (Wang et al., 1994). Among the markers, *RG16*, located on chromosome 11, was also associated with complete resistance to the same rice blast isolate. In addition, three other marker loci associated with partial resistance, *RG64*, *RG869B*, and *RG333*, were found to be linked to the previously mapped *R*-genes *Pi-2(t)*, *Pi-4(t),* and *Pi-zh*, respectively (Yu et al., 1991). The results of this study indicate that more than one resistance gene for rice blast may reside in this region of chromosome 11. In other crops, QTL for *P. infestans* resistance and the dominant race specific allele *R1* have been identified on chromosome V of potato, in the interval formed by markers *GP21* and *GP179* (Leonards-Schippers et al., 1994). The possibility that these QTL are alleles of *R*-genes suggests that the QTL may have a similar function in the resistance mechanism. The fact that some QTL have been discovered to be race-specific (Qi et al., 1999) and involved in HR (Vleeshouwers et al., 2000) also lends support to this theory.

Numerous genes involved in resistance have been isolated and cloned. Sequence analysis has revealed that there exist four major classes of *R*-genes and that their functional domains are highly conserved (Bent, 1996). Degenerate primers, designed from these consensus sequences, are used to amplify *R*-gene analogues (RGAs) in the candidate gene approach. Some of these RGAs have also mapped to regions containing quantitative and/or qualitative resistance loci. A candidate gene approach was used to identify and map QTL for resistance to anthracnose (*Colletotrichum lindemuthianum*) in common bean (Geffroy et al., 2000). Using a RIL population, and candidate genes, 10 QTL for resistance were identified. The candidate genes that were used included pathogen recognition genes, such as *R*-genes and RGAs, and general DR genes. Three of the QTL, linked to marker loci *D1020*, *D1861*, and *D1512*, on linkage groups B3, B7, and B11 respectively, were also associated with previously mapped QTL for resistance to CBB (Nodari et al., 1993). *D1512*, on linkage group B11, is also located in the same genomic region as the qualitative *Co-2* gene for resistance to anthracnose, and a family of leucine-rich repeat (LRR) sequences (Geffroy et al., 1998).

QTL have also been mapped to resistance gene clusters in different crops. Comparative mapping is a strategy that has been increasingly more feasible as more maps are generated across diverse taxa. The fundamental concept is based on the finding that diverse taxa with common taxonomic families often share similar gene order over large chromosomal segments. Therefore, QTL mapped in one species may be located at the same chromosomal region in another evolutionarily related species (Grube et al., 2000). Using comparative mapping, four QTL for resistance to *Erwinia carotovora* ssp. *atroseptica*, mapped to genomic segments in potato containing RGAs, qualitative and quantitative factors conditioning resistance to different pathogens that attack potato, tomato, and tobacco (*Nicotiana tabacum*; Zimnoch-Guzowska et al., 2000). Shared markers between the potato linkage map generated by this study and other potato and tomato maps, were used as anchors to align the maps and allow positional comparisons. *Eca1A*, a major QTL for resistance to *E. carotovora* ssp. *atroseptica*, is located in a similar genomic region in potato as the *Cf* gene family in tomato. The *Cf* genes in tomato are *R*-genes that

confer resistance to the fungal pathogen *Cladosporium fulvum*. The QTL, *Eca6A*, is situated in a genomic segment, which in tomato contains the qualitative genes for nematode (*Meloidogyne* spp.) resistance, *Mi*, and the *Cf-2* gene. In addition, *Eca11A* maps to the same region in potato as another QTL for resistance to *P. infestans*, the virus resistance gene *Ry*, the *Synchytrium endobioticumm* resistance gene, and to the virus resistance *N* gene in tobacco. Several factors may contribute to the clustering effect that has been observed between resistance-related genes (resistance-related includes quantitative, and qualitative genes and RGAs). Clustering could be an anomaly resulting from small population sizes or insufficient number of markers used to precisely map the loci. If the genes were located in an area of reduced recombination rate this would also result in a cluster at that region. Another important factor is that not all resistance-related genes have been identified and mapped, therefore, it is not possible to make absolute conclusions about the clustering of resistance-related genes. The clustering of resistance-related loci in species of the *Solanaceae* family was supported by another comparative mapping study in tomato, potato, and pepper (*Capsicum annuum*) where several cross-generic clusters were observed (Grube et al., 2000).

Most pathologists would agree that the plant developmental stage used to evaluate resistance influences the type of resistance that is detected. Adult plant resistance is generally considered to be quantitative and distinct from the qualitative resistance detected in a seedling assay. Two QTL that confer seedling resistance to three isolates of stripe rust (*Puccinia striiformis* f. sp. *hordei*) in barley mapped to the same region as two of the four QTL that conferred adult plant resistance (Castro et al., 2002). Coincident QTL detected in distinct assays in different plant stages suggest different gene action, yet QTL analysis illustrated colocalization. Linkage mapping of quantitative resistance has revealed other examples of colocalization of major R-genes and QTL for resistance in a wide array of host/pathogen interactions: powdery mildew (*Erysiphe graminis*) in barley (Backes et al., 1996), potyvirus in pepper (Caranta et al., 1997), northern corn leaf blight (*Exserohilum turcicum*) in maize (Freymark et al., 1993), powdery mildew (*Blumeria graminis*) in wheat (Keller et al., 1999), and cyst nematode (*Globodera* spp.) in potato (van der Voort et al., 1998).

QTL may be Defeated Qualitative Resistance Genes

The term defeated, or ghost genes, was first introduced by Riley (1973) to explain the minor contribution to resistance of major genes that were defeated by virulent strains of a pathogen. Defeated genes were visualized as contributing to quantitative resistance controlled by polygenes. Martin and Ellingboe (1976) proposed that defeated major genes may conserve residual resistance effects. They showed that *Pm* genes that had been overcome by virulent isolates of powdery mildew still contributed to the partial resistance in wheat. Nass et al. (1981) also found residual resistance effects for two *Pm* resistance loci, *Pm3c* and *Pm4b*, but not for *Pm2* and *Pm5* loci in wheat. Keller et al. (1999), however, reported that the *Pm5* gene showed a large effect, despite

the use of virulent races of powdery mildew being present in the mixture of isolates used. They concluded that the detected effect of the *Pm5* gene could be explained by the reduced growth of isolates that contain *avrPm5* virulence gene due to reduced fitness and/or the delayed spread of the *Pm5* virulent isolates due to the residual effect of the defeated gene. This residual effect could result from the limited expression of the overcome gene (Nelson, 1978). Li et al. (1999) used a population of 315 RILs and a linkage map that consisted of 182 RFLP markers to map the major gene (*Xa4*) and 10 QTL linked to resistance to bacterial blight, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) in rice. They found that most QTL mapped to genomic regions where major genes or other QTL for *Xoo* resistance were located. In addition, they discovered that the $Xa4^T$ locus, an allele of *Xa4* from the cultivar, "Tequing", behaved as a dominant major resistance gene against strains CR4 and CX08 and as a recessive QTL against strain CR6 of *Xoo*. The resistance conferred by the $Xa4^T$ allele, however, was overcome by the mutation at the *avrXa4* locus in the virulent strain CR6. The *Xa4* gene is considered to be a defeated major resistance gene that plays a key role in rice–*Xoo* interaction (Narayanan et al., 2002). These data suggest that the major genes and QTL for resistance, in this instance, may be the same gene and supports the hypothesis that defeated major resistance genes have residual effects against different races of the same pathogen.

QTL may be Members of Multigene Families

An alternative hypothesis is that QTL and major genes that map to the same genomic region are different members of a cluster of resistance gene families. Many studies have demonstrated that major genes are often members of clustered multigene families (Michelmore and Meyers, 1998; Parniske et al., 1997; Pryor and Ellis, 1993; Ronald, 1998; Song et al., 1997; Yu et al., 1996). These clusters are composed of linked, and evolutionarily related, resistance specificities, therefore, a potential for a structural and functional similarity between qualitative genes and the resistance QTL that map to the same region exists. Grube et al. (2000) showed that major resistance genes can occur in transgeneric clusters with QTL in the *Solanaceae*, suggesting that sequence similarity and probably function similarities exist between qualitative and quantitative genes for resistance. Therefore, QTL that map to the same genomic regions as specific resistance genes may be involved in pathogen recognition. In rice, the major resistance gene *Xa21* is a member of a multigene family located on chromosome 11 that confers race specific resistance to *Xoo*. *Xa21* encodes an extracellular LRR domain and a serine/threonine kinase that is believed to determine the race-specific resistance response (Ronald, 1997). Another member *Xa21D* of the same gene family displays the same resistance spectrum as *Xa21* but confers only partial resistance. *Xa21D* only encodes an extracellular LRR domain due to a retrotransposon insertion. The LRR domain was shown to control race-specific pathogen recognition. This study lends support to the theory that changes in major genes could produce a gene, which confers partial resistance that breeders recognize as quantitative in function.

3.9 Colocalization of QTL with Defense Response Genes

Plant defense response is a complex mechanism that is triggered by pathogen attack. The DR is highly conserved between mono- and dicotyledonous plants and is responsive to different types of pathogens. Numerous DR genes have been cloned (Lamb et al., 1989) and several colocalizations with QTL have been observed, lending support to the hypothesis that colocalization may reflect a functional relationship between the QTL and the DR genes. In common bean, Geffroy et al. (2000) mapped a QTL for stem resistance against *C. lindemuthianum* strain A7 of bean anthracnose near a locus for phenylalanine ammonia-lyase (*Pal-2*). This enzyme is a critical branch point control for the biosynthetic pathways of certain antimicrobial phenolic compounds. Another QTL was mapped on linkage group B7 near the hydroxyproline-rich glycoprotein locus, *Hrgp36*. These types of proteins are believed to contribute to the formation of a structural barrier to block pathogen invasion. The researchers conclude that allelic variants of *Pal-2* and *Hgp36* may be responsible for the differences in quantitative resistance to *C. lindemuthianum*. This evidence supports the theory that molecular polymorphisms within the DR genes result in allelic diversity and may relate to differences in resistance levels (Pflieger et al., 2001). Pflieger et al. (2001) mapped several DR genes to genomic regions corresponding with QTL for resistance to different pathogens in pepper. A class-III chitinase gene colocalized with a QTL conferring resistance to *Phytophthora capsici* in pepper. In addition, three pathogenesis-related protein (PR) loci mapped within the region containing QTL to *P. capsici*, Potato virus Y, and potyvirus E in pepper.

QTL located on linkage group B2 of the common bean map (Freyre et al., 1998; Kelly et al., 2003) spanned a region that encompasses the *Pv*PR2 locus, and suggested a role for this PR protein in resistance to *Fusarium* root rot and white mold in common bean (Kolkman and Kelly, 2003; Schneider et al., 2001). Defense response genes, such as the *P. vulgaris* pathogenesis-related gene, *PvPR-2* (Walter et al., 1990), a polygalacturonase-inhibiting protein, *Pgip* (Toubart et al., 1992), and the chalcone synthase gene, *ChS* (Ryder et al., 1987) located on B2 invites speculation that fungal defense-related genes are triggered as a general resistance response to *Fusarium* and *Sclerotinia* infection, suggesting that physiological resistance is associated with a generalized host defense response. *Pv*PR2 and its counterpart *Pv*PR1 are low molecular weight acidic proteins induced during fungal elicitation (Walter et al., 1990). These bean PR proteins share similarities with PR proteins in crops such as potato, parsley (*Petroselinum crispum*), and pea (*Pisum sativum*). Linkage was also reported between QTL conferring partial resistance to Ascochyta blight (*Mycosphaerella pinodes*) of pea and candidate genes including DR and RGA located on the pea linkage map (Timmerman-Vaughan et al., 2002). The role of *Pv*PR proteins in *Fusarium* resistance in common bean is further confirmed by the significant association observed between QTL that map to B3 in the region of *Pv*PR1 gene. Differences in *Pv*PR gene arrangements were detected between anthracnose (*C. lindemuthianum*) resistant and susceptible

bean genotypes indicating that polymorphism between *Pv*PR as well as other defense response-related genes may contribute to our understanding of quantitative resistance (Walter et al., 1990). QTL associated with resistance to the late blight fungus of potato have also been reported to colocalize with DR genes for specific PR proteins in potato (Gebhardt et al., 1991; Leonards-Schippers et al., 1994). To capitalize on the assumption that defense proteins may be associated with quantitative resistance, a method of candidate gene analysis where genes known to be involved in host defense responses are used as markers to identify potential QTL associated with disease resistance, has been evaluated in maize (Byrne et al., 1996; Causse et al., 1995; Goldman et al., 1993) and wheat (Faris et al., 1999), and could be employed to improve root rot and white mold resistance in common bean.

In summary, there appears to be two kinds of coincident QTL: those that map with major genes and those that map with DR genes. The QTL that map with major genes could be allelic versions of those genes. Some of those alleles may confer partial resistance rather than complete resistance as a result of a mutation in the pathogen that now overcomes the original resistance gene. In the other instance, QTL that map with major genes may also be members of a multigene family that is involved with recognition of the pathogen. QTL that map with DR genes may be involved in a general defense mechanism. To differentiate between the various hypotheses, fine mapping of the region containing the QTL is needed to determine if the relationship between the QTL and the colocalized gene is allelic or not.

3.10 Conclusions

In a computer simulation study, Bernardo (2001) concluded that genomics is of limited value in the selection for quantitative traits in hybrid (and self-pollinated) crops. Despite this dire prognosis, Bernando (2001) stated that gene information (we equate "gene information" with "QTL analysis") is most useful in selection when fewer than 10 loci control the trait and becomes imprecise when the number exceeds 50 loci. Although the actual number of loci controlling multigenic disease resistance is not generally known, the number is most likely to be under 10 than exceed 50 loci, and therefore, be responsive to selection using QTL analysis. Performance based traits are more likely to exceed 50 loci than those conditioning quantitative disease resistance (Young, 1996). QTL analysis provides plant breeders with the tools to reassemble, into future cultivars, the multigenes influencing quantitative resistance, previously not possible through routine disease screening. Unlike qualitative resistance, where genotype and phenotype are one and the same, breeders struggle with reassembling, in new cultivars, all the genes that controlled quantitative resistance after they were "disassembled" in crossing. The breeding literature is fraught with examples of the partial recovery of quantitative resistance from unique genetic stocks. With the knowledge of the location and size of the QTL controlling quantitative resistance breeders can use MAS to reassemble

that resistance in new genetic backgrounds and restore it to levels present in the original sources. Due to the complexity of quantitative resistance, breeders often failed to adequately compare resistance sources for uniqueness. QTL analysis provides breeders with a tool to compare the location and size of individual effects to determine if new resistance sources are unique prior to undertaking the long and arduous process of utilizing quantitative resistance in breeding. One area where QTL analysis offers exciting opportunities is in the utilization of wild germplasm in resistance breeding. Prebreeding using markers linked to resistance traits to introgress genomic regions from the wild to cultivated species is being investigated in many crops using breeding methods such as the advanced-backcross QTL analysis (Tanksley and McCouch, 1997). Combining backcrossing with MAS allows breeders to evaluate the potential of specific genomic regions from the wild species in the genetic background of the cultivated species, as the expression of quantitative resistance in the field can only be tested in adapted lines.

Aside from the practical utility and efficiency that QTL analysis brings to breeding of quantitative resistance, the identification and location of QTL is providing new insights to many of the age-old theories and controversies that have competed for importance in the literature. Many of these theories have impeded the process of resistance breeding as they classified resistance into two clear camps, implying that selection for one form of resistance would impede the use of the other. New information is being provided by QTL analyses on such questions as: (1) the actual durability of quantitative resistance sources, (2) the possible distinction between the resistance detected by seedling assays and adult plant resistance, (3) the potential of defeated major genes in resistance breeding, (4) the actual similarities between qualitative and quantitative resistance sources, (5) the nature of the differences in resistance may reside in expression due to interaction with genetic backgrounds or pathotypes, and (6) the opportunity to clone underlying genetic factors that confer quantitative resistance as was demonstrated for fruit size in tomato (Frary et al., 2000).

Genetic mapping, in general, has provided breeders with new insights into old problems. The evidence that resistance gene clusters exist in plants is widely reported (Michelmore and Meyers, 1998), so why should qualitative and quantitative resistance mechanisms be different? Resistance gene clusters imply that in the plant the DR genes are localized and can be shared in response to attack by different pathogens and/or stress factors. QTL analyses are adding to the body of evidence that in many instances qualitative and quantitative resistance reside in the same regions and are differential responses to different pathotypes, and the methods used by scientists to detect and measure their effect. For example, the literature is fraught with implications that seedling resistance is qualitative and adult plant resistance is more complex, hence quantitative and distinct. QTL for seedling resistance to stripe rust in barley has been shown to share common QTL for adult plant resistance (Castro et al., 2002). Defeated major resistance genes are receiving renewed attention as QTL analyses position partial or quantitative resistance in those regions of the genome where major genes reside (Li et al., 1999). The importance of defeated genes in resistance breeding is obvious as the

underlying suggestion that breeders are not always finding unique or new sources of resistance but differential expression of existing sources. Breeders may need to consider how best to utilize existing resistance sources rather than search for new sources that may prove to be elusive. If quantitative resistance is distinct from major *R*-genes functional in a specific crop/pathogen system, there is an increasing body of evidence that supports the role of DR genes in quantitative resistance. DR genes have been shown to play a key role in resistance (Lamb et al., 1989) but their effect is only partial, not unlike the effect(s) that defines quantitative resistance (Parlevliet, 1975). QTL analyses are placing partial resistance sources in genomic regions where DR genes are located (Pflieger et al., 2001). The partial resistance detected in QTL analysis may not be due to actual resistance genes similar to *R*-genes but may be due to an enhanced expression of DR genes (Schneider et al., 2001). The role that DR genes can enhance resistance is known, so links between quantitative resistance and DR genes is interesting and could benefit breeders as DR genes have similar functionality across plant species.

Finally, mapping studies have shown that cereal genomes exhibit a high degree of synteny (Gale and Devos, 1998). Based on this information, QTL for resistance to Fusarium head blight (*Fusarium graminearum*) in wheat and barley appear to reside in syntenous locations on chromosome 3 in both crops (Kolb et al., 2001). Breeders can use the conservation of gene order and position among related species to assist in the identification of resistance sources that may be absent from their crop. QTL markers could be used to probe for resistance in one species based on the presence of QTL for resistance in a related species and provide breeders with the opportunity to use alternative resistance sources in the development of future cultivars with adequate levels of disease resistance.

References

Ali, M.A. 1950. Genetics of resistance to the common bean mosaic virus bean virus 1) in the bean (*Phaseolus vulgaris* L.). *Phytopathology* 40:69–79.

Ariyarathne, H.M., Coyne, D.P., Jung, G., Skroch P.W., Vidaver, A.K., Steadman, J.R., Miklas, P.N., and Bassett,. M.J. 1999. Molecular mapping of disease resistance genes for halo blight, common bacterial blight, and bean common mosaic virus in a segregating population of common bean. *J. Am. Soc. Horticult. Sci.* 124:654–662.

Asins, M.J. 2002. Present and future of quantitative trait locus analysis in plant breeding. *Plant Breed.* 121:281–291.

Backes, G., G. Schwarz, Wenzel, G., and Jahoor, A. 1996. Comparison between QTL analysis of powdery mildew resistance in barley based on detached primary leaves and on field data. *Plant Breed.* 115:419–421.

Bent, A.F. 1996. Plant disease resistance genes: function meets structure. *Plant Cell* 8:1757–1771.

Bernardo, R. 2001. What if we knew all the genes for a quantitative trait in hybrid crops? *Crop Sci.* 41:1–4.

Botstein, D., White, R.L., Skolnick, M., and Davis, R.W. 1980. Construction of a genetic-linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32:314–331.

Burr, B., and Burr, F.A. 1991. Recombinant inbreds for molecular mapping in maize – theoretical and practical considerations. *Trends Genet*. 7:55–60.

Byrne, P.F., McMullen, M.D., Snook, M.E., Musket, T.A., Theuri, J.M., Widstrom, N.W., Wiseman, B.R., and Coe, E.H. 1996. Quantitative trait loci and metabolic pathways: Genetic control of the concentration of maysin, a corn earworm resistance factor, in maize silks. *Proc. Natl. Acad. Sci. USA* 93:8820–8825.

Caranta, C., Lefebvre, V., and Palloix, A. 1997. Polygenic resistance of pepper to potyviruses consists of a combination of isolate-specific and broad-spectrum quantitative trait loci. *Mol. Plant Microbe Interact*. 10:872–878.

Castano, F., Vear, F., and Delabrouhe, D.T. 1993. Resistance of sunflower inbred lines to various forms of attack by *Sclerotinia sclerotiorum* and relations with some morphological characters. *Euphytica* 68:85–98.

Castro, A.J., Chen, X.M., Hayes, P.M., Knapp, S.J., Line, R.F., Toojinda, T., and Vivar, H. 2002. Coincident QTL which determine seedling and adult plant resistance to stripe rust in barley. *Crop Sci*. 42:1701–1708.

Causse, M., Rocher, J.P., Henry, A.M., Charcosset, A., Prioul, J.L., and Devienne, D. 1995. Genetic dissection of the relationship between carbon metabolism and early growth in maize, with emphasis on key- enzyme loci. *Mol. Breed*. 1:259–272.

Chen, F.Q., Prehn, D., Hayes, P.M., Mulrooney, D., Corey, A., and Vivar, H. 1994. Mapping genes for resistance to barley stripe rust (*Puccinia striiformis* f. sp. *hordei*). *Theor. Appl. Genet*. 88:215–219.

Coyne, D.P. 1980. Modification of plant architecture and crop yield by breeding. *HortScience* 15:244–247.

Cregan, P.B., Mudge, J., Fickus, E.W., Marek, L.F., Danesh, D., Denny, R., Shoemaker, R.C., Matthews, B.F., Jarvik, T., and Young, N.D. 1999a. Targeted isolation of simple sequence repeat markers through the use of bacterial artificial chromosomes. *Theor. Appl. Genet*. 98:919–928.

Cregan, P.B., Jarvik, T., Bush, A.L., Shoemaker, R.C., Lark, K.G., Kahler, A.L., Kaya, N., VanToai, T.T., Lohnes, D.G., Chung, L., and Specht, J.E. 1999b. An integrated genetic linkage map of the soybean genome. *Crop Sci*. 39:1464–1490.

Danesh, D., and Young, N.D. 1994. Partial resistance loci for tomato bacterial wilt show differential race specificity. *Rep. Tomato Genet. Coop*. 44:12–13.

Eenink, A.H. 1976. Genetics of host-parasite relationships and uniform and differential resistance. *Neth. J. Plant Pathol*. 82:133–145.

Faris, J.D., Li, W.L., Liu, D.J., Chen, P.D., and Gill, B.S. 1999. Candidate gene analysis of quantitative disease resistance in wheat. *Theor. Appl. Genet*. 98:219–225.

Frary, A., Nesbitt, T.C., Grandillo, S., van der Knaap, E., Cong, B., Liu, J.P., Meller, J., Elber, R., Alpert, K.B., and Tanksley, S.D. 2000. Fw2.2: a quantitative trait locus key to the evolution of tomato fruit size. *Science* 289:85–88.

Freymark, P.J., Lee, M., Woodman, W.L., and Martinson, C.A. 1993. Quantitative and qualitative trait loci affecting host-plant response to *Exserohilum turcicum* in maize (*Zea mays* L). *Theor. Appl. Genet*. 87:537–544.

Freyre, R., Skroch, P.W., Geffroy, V., Adam-Blondon, A.F., Shirmohamadali, A., Johnson, W.C., Llaca, V., Nodari, R.O., Pereira, P.A., Tsai, S.M., Tohme, J., Dron, M., Nienhuis, J., Vallejos, C.E., and Gepts, P. 1998. Towards an integrated linkage map of common bean. 4. Development of a core linkage map and alignment of RFLP maps. *Theor. Appl. Genet*. 97:847–856.

Gale, M.D., and Devos, K.M. 1998. Plant comparative genetics after 10 years. *Science* 282:656–659.

Gebhardt, C., Ritter, E., Barone, A., Debener, T., Walkemeier, B., Schachtschabel, U., Kaufmann, H., Thompson, R.D., Bonierbale, M.W., Ganal, M.W., Tanksley, S.D., and Salamini, F. 1991. RFLP maps of potato and their alignment with the homoeologous tomato genome. *Theor. Appl. Genet*. 83:49–57.

Geffroy, V., Creusot, F., Falquet, J., Sevignac, M., Adam-Blondon, A.F., Bannerot, H., Gepts, P., and Dron, M. 1998. A family of LRR sequences in the vicinity of the *Co-2* locus for anthracnose resistance in *Phaseolus vulgaris* and its potential use in marker-assisted selection. *Theor. Appl. Genet*. 96:494–502.

Geffroy, V., Sevignac, M., De Oliveira, J.C.F., Fouilloux, G., Skroch, P., Thoquet, P., Gepts, P., Langin, T., and Dron, M. 2000. Inheritance of partial resistance against *Colletotrichum lindemuthianum* in *Phaseolus vulgaris* and co-localization of quantitative trait loci with genes involved in specific resistance. *Mol. Plant Microbe Interact*. 13:287–296.

Giovannoni, J.J., Wing, R.A., Ganal, M.W., and Tanksley, S.D. 1991. Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. *Nucleic Acids Res*. 19:6553–6558.

Goldman, I.L., Rocheford, T.R., and Dudley, J.W. 1993. Quantitative trait loci influencing protein and starch concentration in the Illinois long-term selection maize strains. *Theor. Appl. Genet*. 87:217–224.

Grube, R.C., Radwanski, E.R., and Jahn, M. 2000. Comparative genetics of disease resistance within the *Solanaceae*. *Genetics* 155:873–887.

Hadley, H.H., and Openshaw, S.J. 1980. Interspecific and intergeneric hybridization. In *Hybridization of Crop Plants*, eds. W.R. Fehr, and H.H. Hadley, pp. 133–159. Amdison, WI: American Society Agronomy.

Hallauer, A.R., and Miranda, J.B. 1981. *Quantitative Genetics in Maize Breeding*. Ames IA: Iowa State University Press.

Hammond-Kosack, K.E., and Jones, J.D.G. 1997. Plant disease resistance genes. *Ann. Rev. Plant Phys. Plant Mol. Biol*. 48:575–607.

Harrison, B.D. 2002. Virus variation in relation to resistance-breaking in plants. *Euphytica* 124:181–192.

Honma, S. 1956. A bean interspecific hybrid. *J. Hered*. 47:217–220.

Hunter, R.L., and Markert, C.L. 1957. Histochemical demonstration of enzymes separated by zone electrophoreses in starch gels. *Science* 125:1294–1295.

Jensen, J. 1989. Estimation of recombination parameters between a quantitative trait locus (QTL) and two marker gene loci. *Theor. Appl. Genet*. 78:613–618.

Johnson, R. 1984. A critical analysis of durable resistance. *Annu. Rev. Phytopathol*. 22:309–330.

Jones, E.S., Breese, W.A., Liu, C.J., Singh, S.D., Shaw, D.S., and Witcombe, J.R. 2002. Mapping quantitative trait loci for resistance to downy mildew in pearl millet: Field and glasshouse screens detect the same QTL. *Crop Sci*. 42:1316–1323.

Jung, G., Skroch, P.W., Nienhuis, J., Coyne, D.P., Arnaud-Santana, E., Ariyarathne, H.M., and Marita, J.M. 1999. Confirmation of QTL associated with common bacterial blight resistance in four different genetic backgrounds in common bean. *Crop Sci*. 39:1448–1455.

Keller, M., Keller, B., Schachermayr, G., Winzeler, M., Schmid, J.E., Stamp, P., and Messmer, M.M. 1999. Quantitative trait loci for resistance against powdery mildew in a segregating wheat x spelt population. *Theor. Appl. Genet*. 98:903–912.

Kelly, J.D., Gepts, P., Miklas, P.N., and Coyne, D.P. 2003. Tagging and mapping of genes and QTL and molecular marker-assisted selection for traits of economic importance in bean and cowpea. *Field Crops Res*. 82:135–154.

Kelly, J.D., and V.A. Vallejo. 2005. QTL analysis of multigenic disease resistance in plant breeding. *In* S. Tuzun and E. Bent (ed.) Multigenic and Induced Systemic Resistance in Plants (in press).

Kim, H.S., and Diers, B.W. 2000. Inheritance of partial resistance to Sclerotinia stem rot in soybean. *Crop Sci*. 40:55–61.

Knapp, S.J. 1991. Using molecular markers to map multiple quantitative trait loci - models for backcross, recombinant inbred, and doubled haploid progeny. *Theor. Appl. Genet*. 81:333–338.

Knapp, S.J., Bridges, W.C., and Birkes. D. 1990. Mapping quantitative trait loci using molecular marker linkage maps. *Theor. Appl. Genet*. 79:583–592.

Kolb, F.L., Bai, G.H., Muehlbauer, G.J., Anderson, J.A., Smith, K.P., and Fedak, G. 2001. Host plant resistance genes for fusarium head blight: mapping and manipulation with molecular markers. *Crop Sci*. 41:611–619.

Kolkman, J.M., and Kelly, J.D. 2002. Agronomic traits affecting resistance to white mold in common bean. *Crop Sci*. 42:693–699.

Kolkman, J.M., and Kelly, J.D. 2003. QTL conferring resistance and avoidance to white mold in common bean. *Crop Sci*. 43:539–548.

Lamb, C.J., Lawton, M.A., Dron, M., and Dixon, R.A. 1989. Signals and transduction mechanisms for activation of plant defenses against microbial attack. *Cell* 56:215–224.

Lander, E.S., and Botstein, D. 1986a. Mapping complex genetic-traits in humans: new methods using a complete RFLP linkage map. *Cold Spring Harb. Symp. Quant. Biol*. 51:49–62.

Lander, E.S., and Botstein, D. 1986b. Strategies for studying heterogeneous genetic-traits in humans by using a linkage map of restriction fragment length polymorphisms. *Proc. Natl. Acad. Sci. USA* 83:7353–7357.

Lander, E.S., and Botstein, D. 1989. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121:185–199.

Leonards-Schippers, C., Gieffers, W., Schaferpregl, R., Ritter, E., Knapp, S.J., Salamini, F., and Gebhardt, C. 1994. Quantitative resistance to *Phytophthora infestans* in potato – a case-study for QTL mapping in an allogamous plant-species. *Genetics* 137: 67–77.

Li, Z.K., Luo, L.J., Mei, H.W., Paterson, A.H., Zhao, X.Z., Zhong, D.B., Wang, Y.P., Yu, X.Q., Zhu, L., Tabien, R., Stansel, J.W., and Ying, C.S. 1999. A "defeated" rice resistance gene acts as a QTL against a virulent strain of *Xanthomonas oryzae* pv. o*ryzae*. *Mol. Gen. Genet*. 261:58–63.

Lindhout, P. 2002. The perspectives of polygenic resistance in breeding for durable disease resistance. *Euphytica* 124:217–226.

Liu, B.H. 1998. *Statistical genomics: Linkage, Mapping, and QTL Analysis*. Boca Raton, FL: CRC Press.

Lubberstedt, T., Klein, D., and Melchinger, A.E. 1998. Comparative QTL mapping of resistance to *Ustilago maydis* across four populations of European flint-maize. *Theor. Appl. Genet*. 97:1321–1330.

Mangin, B., Thoquet, P., Olivier, J., and Grimsley, N.H. 1999. Temporal and multiple quantitative trait loci analyses of resistance to bacterial wilt in tomato permit the resolution of linked loci. *Genetics* 151:1165–1172.

Martin, T.J., and Ellingboe, A.H. 1976. Differences between compatible parasite/host genotypes involving the *Pm4* locus of wheat and the correspondiing genes in *Erysiphe graminis* f. sp. *tritici*. *Phytopathology* 66:1435–1438.

McElroy, J.B. 1985. Breeding dry beans, *Phaseolus vulgaris* L. for common bacterial blight resistance derived from *Phaseolus acutifolius* A. Gray. Ph.D. dissertation., Cornell University, Ithaca, N.Y.

Mestries, E., Gentzbittel, L., de Labrouhe, D.T., Nicolas, P., and Vear, F. 1998. Analyses of quantitative trait loci associated with resistance to *Sclerotinia sclerotiorum* in sunflowers (*Helianthus annuus* L.), using molecular markers. *Mol. Breed.* 4:215–226.

Michelmore, R.W., and Meyers, B.C. 1998. Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. *Genome Res.* 8:1113–1130.

Michelmore, R.W., Paran, I., and Kesseli, R.V. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci. USA* 88:9828–9832.

Miklas, P.N., Stone, V., Urrea, C.A., Johnson, E., and Beaver, J.S. 1998. Inheritance and QTL analysis of field resistance to ashy stem blight in common bean. *Crop Sci.* 38:916–921.

Miklas, P.N., Johnson, E., Stone, V., Beaver, J.S., Montoya, C., and Zapata, M. 1996. Selective mapping of QTL conditioning disease resistance in common bean. *Crop Sci.* 36:1344–1351.

Miklas, P.N., Smith, J.R., Riley, R., Grafton, K.F., Singh, S.P., Jung, G., and Coyne, D.P. 2000. Marker-assisted breeding for pyramided resistance to common bacterial blight in common bean. *Ann. Rep. Bean Improv. Coop.* 43:39–40.

Miklas, P.N., Coyne, D.P., Grafton, K.F., Mutlu, N., Reiser, J., Lindgren, D.T., and Singh, S.P. 2003. A major QTL for common bacterial blight resistance derives from the common bean great northern landrace cultivar Montana No. 5. *Euphytica* 131:137–146.

Mundt, C.C., Cowger, C., and Garrett, K.A. 2002. Relevance of integrated disease management to resistance durability. *Euphytica* 124:245–252.

Narayanan, N.N., Baisakh, N., Vera Cruz, C.M., Gnanamanickam, S.S., Datta, K., and Datta, S.K. 2002. Molecular breeding for the development of blast and bacterial blight resistance in rice cv. IR50. *Crop Sci* 42:2072–2079.

Nass, H.A., Pedersen, W.L., Mackenzie, D.R., and Nelson, R.R. 1981. The residual effects of some "defeated" powdery mildew resistance genes in isolines of winter wheat. *Phytopathology* 71:1315–1318.

Nelson, R.R. 1978. Genetics of horizontal resistance to plant diseases. *Annu. Rev. Phytopathol.* 16:359–378.

Nodari, R.O., Tsai, S.M., Guzman, P., Gilbertson, R.L., and Gepts, P. 1993. Toward an integrated linkage map of common bean. 3. Mapping genetic-factors controlling host-bacteria interactions. *Genetics* 134:341–350.

Parlevliet, J.E. 1975. Disease resistance in plants and its consequences for plant breeding, *In Plant Breeding II*, ed K.J. Frey., pp. 309–364. Ames, IA: Iowa State University Press.

Parlevliet, J.E. 2002. Durability of resistance against fungal, bacterial and viral pathogens; present situation. *Euphytica* 124:147–156.

Parlevliet, J.E., and Zadoks, J.C. 1977. Integrated concept of disease resistance - new view including horizontal and vertical resistance in plants. *Euphytica* 26:5–21.

Parniske, M., Hammond-Kosack, K.E., Golstein, C., Thomas, C.M., Jones, D.A., Harrison, K., Wulff, B.B.H., and Jones, J.D.G. 1997. Novel disease resistance specificities result from sequence exchange between tandemly repeated genes at the *Cf-4/9* locus of tomato. *Cell* 91:821–832.

Paterson, A.H. 1998. Of blending, beans, and bristles: the foundations of QTL mapping, In *Molecular Dissection of Complex Traits*, ed. A.H. Paterson, pp. 1–10. Boca Raton, FL: CRC Press.

Pflieger, S., Palloix, A., Caranta, C., Blattes, A., and Lefebvre, V. 2001. Defense response genes co-localize with quantitative disease resistance loci in pepper. *Theor. Appl. Genet.* 103:920–929.

Pilet, M.L., Delourme, R., Foisset, N., and Renard, M. 1998. Identification of QTL involved in field resistance to light leaf spot (*Pyrenopeziza brassicae*) and blackleg resistance (*Leptosphaeria maculans*) in winter rapeseed (*Brassica napus* L.). *Theor. Appl. Genet.* 97:398–406.

Pryor, T., and Ellis, J. 1993. The genetic complexity of fungal resistance genes in plants. *Adv. Plant Pathol.* 10:281–305.

Qi, X., Niks, R.E., Stam, P., and Lindhout, P. 1998. Identification of QTLs for partial resistance to leaf rust (*Puccinia hordei*) in barley. *Theor. Appl. Genet.* 96:1205–1215.

Qi, X., Jiang, G., Chen, W., Niks, R.E., Stam, P., and Lindhout, P. 1999. Isolate-specific QTLs for partial resistance to *Puccinia hordei* in barley. *Theor. Appl. Genet.* 99: 877–884.

Riley, R. 1973. Genetic changes in hosts and the significance of disease. *Ann. Appl. Biol.* 75:128–132.

Robertson, D.S. 1989. Understanding the relationship between qualitative and quantitative genetics, In *Development and Application of Molecular Markers to Problems in Plant Genetics*, eds. T. Helentjaris, and B. Benjamin, pp. 81–87. Cold Spring, Harbor, NY: Cold Spring Harbor Laboratory.

Ronald, P.C. 1997. The molecular basis of disease resistance in rice. *Plant Mol. Biol.* 35:179–186.

Ronald, P.C. 1998. Resistance gene evolution. *Curr. Opin. Plant Biol.* 1:294–298.

Ronin, Y.I., Korol, A.B., and Weller, J.I. 1998. Selective genotyping to detect quantitative trait loci affecting multiple traits: interval mapping analysis. *Theor. Appl. Genet.* 97:1169–1178.

Ryder, T.B., Hedrick, S.A., Bell, J.N., Liang, X.W., Couse, S.D., and Lamb, C.J. 1987. Organization and differential activation of a gene family encoding the plant defense enzyme chalcone synthase in *Phaseolus vulgaris*. *Mol. Gen. Genet.* 210:219–233.

Sax, K. 1923. The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. *Genetics* 8:552–560.

Schechert, A.W., Welz, H.G., and Geiger, H.H. 1999. QTL for resistance to *Setosphaeria turcica* in tropical African maize. *Crop Sci.* 39:514–523.

Schneider, K.A., Grafton, K.F., and Kelly, J.D. 2001. QTL analysis of resistance to fusarium root rot in bean. *Crop Sci.* 41:535–542.

Scott, M.E., and Michaels, T.E. 1992. *Xanthomonas* resistance of *Phaseolus* interspecific cross selections confirmed by field performance. *HortScience* 27:348–350.

Singh, S.P., and Munoz, C.G. 1999. Resistance to common bacterial blight among *Phaseolus* species and common bean improvement. *Crop Sci.* 39:80–89.

Smithies, O. 1955. Zone electrophoreses in starch gels. *Biochem. J.* 61:629.

Song, W.Y., Pi L.Y, Wang, G.L., Gardner, J., Holsten, T., and Ronald, P.C. 1997. Evolution of the rice *Xa21* disease resistance gene family. *Plant Cell* 9:1279–1287.

Sprague, G.F. 1966. Quantitative genetics in plant improvement. In *Plant Breeding*, ed. K.J. Frey, pp. 315–357. Ames, IA: Iowa State University Press.

Staub, J.E., Serquen, F.C., and Gupta, M. 1996. Genetic markers, map construction, and their application in plant breeding. *HortScience* 31:729–741.

Tanksley, S.D., and McCouch, S.R. 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. *Science* 277:1063–1066.

Tanksley, S.D., Young, N.D., Paterson, A.H., and Bonierbale, M.W. 1989. RFLP mapping in plant-breeding - new tools for an old science. *Bio/Technology* 7:257–264.

Thoday, J.M. 1961. Location of polygenes. *Nature* 191:368–370.

Timmerman-Vaughan, G.M., Frew, T.J., Russell, A.C., Khan, T., Butler, R., Gilpin, M., Murray, S., and Falloon, K. 2002. QTL mapping of partial resistance to field epidemics of ascochyta blight of pea. *Crop Sci* 42:2100–2111.

Toubart, P., Desiderio, A., Salvi, G., Cervone, F., Daroda, L., Delorenzo, G., Bergmann, C., Darvill, A.G., and Albersheim, P. 1992. Cloning and characterization of the gene encoding the endopolygalacturonase-inhibiting protein (PGIP of *Phaseolus vulgaris* L. *Plant J*. 2:367–373.

van der Plank, J.E. 1968. *Disease Resistance in Plants*. Academic Press, New York.

van der Voort, J.R., Lindeman, W., Folkertsma, R., Hutten, R., Overmars, H., van der Vossen, E., Jacobsen, E., and Bakker, J. 1998. A QTL for broad-spectrum resistance to cyst nematode species (*Globodera* spp.) maps to a resistance gene cluster in potato. *Theor. Appl. Genet*. 96:654–661.

Vleeshouwers, V., van Dooijeweert, W., Govers, F., Kamoun, S., and Colon, L.T. 2000. The hypersensitive response is associated with host and nonhost resistance to *Phytophthora infestans*. *Planta* 210:853–864.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Vandelee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP – a new technique for DNA-fingerprinting. *Nucleic Acids Res*. 23:4407–4414.

Walter, M.H., Liu, J., Grand, C., Lamb, C.J., and Hess, D. 1990. Bean pathogenesis-related (PR) proteins deduced from elicitor-induced transcripts are members of a ubiquitous new class of conserved PR proteins including pollen allergens. *Mol. Gen. Genet*. 222:353–360.

Wang, G.L., and Paterson, A.H. 1994. Assessment of DNA pooling strategies for mapping of QTL. *Theor. Appl. Genet*. 88:355–361.

Wang, G.L., Mackill, D.J., Bonman, J.M., McCouch, S.R., Champoux, M.C., and Nelson, R.J. 1994. RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. *Genetics* 136:1421–1434.

Weber, J.L., and May, P.E. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain-reaction. *Am. J. Hum. Genet*. 44:388–396.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic-markers. *Nucleic Acids Res*. 18:6531–6535.

Young, N.D. 1996. QTL mapping and quantitative disease resistance in plants. *Annu. Rev. Phytopathol*. 34:479–501.

Yu, Y.G., Buss, G.R., and Maroof, M.A.S. 1996. Isolation of a superfamily of candidate disease-resistance genes in soybean based on a conserved nucleotide binding site. *Proc. Natl. Acad. Sci. USA* 93:11751–11756.

Yu, Z.H., Mackill, D.J., Bonman, J.M., and Tanksley, S.D. 1991. Tagging genes for blast resistance in rice via linkage to RFLP markers. *Theor. Appl. Genet*. 81:471–476.

Zeng, Z.B. 1993. Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. *Proc. Natl. Acad. Sci. USA* 90:10972–10976.

Zeng, Z.B. 1994. Precision mapping of quantitative trait loci. *Genetics* 136:1457–1468.

Zimnoch-Guzowska, E., Marczewski, W., Lebecka, R., Flis, B., Schafer-Pregl, R., Salamini, F., and Gebhardt, C. 2000. QTL analysis of new sources of resistance to *Erwinia carotovora* ssp. *atroseptica* in potato done by AFLP, RFLP, and resistance-gene-like markers. *Crop Sci*. 40:1156–1167.