# Tools for marking plant disease and pest resistance genes : a review

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# Tools for marking plant disease and pest resistance genes: a review

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**Summary** — Genetic mapping of disease resistance genes will help improve the efficiency of plant breeding and will lead to a better understanding of the molecular basis of resistance. It requires, however, both reliable pathological tests and polymorphic genetic markers in a well-defined segregating population. In recent years, a number of different types of genetic markers have become available. The characteristics and gene-tagging efficiency of morphological and molecular markers are reviewed. Two strategies for developing markers for disease resistance genes are presented: the establishment of genetic maps with localization of major genes and QTLs; and targeting particular regions. We discuss how molecular mapping studies provide new insights on the localization and organization of the genes involved in disease resistance.

**genetics / marker / mapping / disease / resistance**

**Résumé — Marquage des gènes de résistance aux parasites chez les végétaux.** *La cartographie génétique des gènes de résistance devrait permettre d'aboutir à une amélioration de l'efficacité des schémas de sélection et de mieux connaître les bases moléculaires de la résistance. Elle nécessite à la fois des tests pathologiques fiables et des marqueurs génétiques polymorphes dans une population en ségrégation bien définie. De nombreux marqueurs génétiques sont désormais disponibles. Les caractéristiques et l'intérêt des marqueurs morphologiques et moléculaires sont décrits. Deux stratégies pour cartographier des régions cibles sont présentées : d'une part la localisation de gènes majeurs ou de QTLs grâce à l'établissement de cartes génétiques, d'autre part l'étiquettage direct de régions particulières. L'apport des études de cartographie sur la connaissance de la localisation et l'organisation des gènes impliqués est discuté.*

***génétique / marqueur / cartographie / maladie / résistance***

* Correspondence and reprints

## CONTENTS

## INTRODUCTION

Breeders, pathologists or entomologists will find information in this paper on the different tools used for mapping plant disease and pest resistance genes, and the most significant data obtained in this field in the last 5–10 years.

Breeding for disease resistance has greatly contributed to improving quality and yield in most crop plants and has led to the identification of genes involved in the expression of resistance and to their recombination through hybridization. These advances were in part the result of studies of pathogen variability and epidemiology. Plant pathologists and breeders have developed simple and reliable test procedures for several major crop diseases. However, diagnostic tests for insects or soil-borne pathogens, such as nematodes, are often difficult to develop due to the challenge posed by inoculum production and maintenance. Moreover, exotic pathogens may require a quarantine period or costly devices to prevent their release in the environment. Testing pathotypes, races or several pathogens on the same plant may be difficult because tests on detached organs are often not possible. In addition, selecting for disease resistance expressed at the adult plant stage is often expensive and difficult to perform.

Even though biological tests will always be needed to confirm marker-assisted selection, their utilization could be reduced by the use of molecular markers, which may greatly accelerate selection programs. Markers could also be powerful tools for genetic analysis and could supply complementary information to classical genetic analyses. The development and usefulness of markers for disease resistance relies, however, on the accuracy of the biological assays which must be sufficiently discriminating to characterize the various components of a quantitative resistance (for instance, a qualitative assay, such as resistance *vs* susceptibility, cannot lead to the identification of several quantitative trait loci). The understanding of the pattern of inheritance will determine the strategy to adopt in developing markers and the type and the size of the progeny to study. The origin of the resistance genes, either intra- or interspecific, will also influence the type of material to study.

Tight linkage between molecular markers and genes for disease resistance can be of great benefit to disease resistance breeding programs by allowing the investigator to follow the DNA markers through the generations rather than waiting for phenotypic expression of the resistance genes. Once a gene has been found to be linked to codominant markers, plants that are heterozygous for a resistance gene can be easily identified; it is therefore possible to introgress recessive or dominant disease resistance genes in a minimum number of generations (Tanksley, 1983; Young and Tanksley, 1989; Tanksley *et al*, 1989). The development of markers also allows the cumulation of several genes in multiresistant genotypes with durable resistance (multigenic resistance) and the analysis of polygenic resistance (Melchinger, 1990). Finally, DNA markers for disease resistance genes may be the starting point for cloning the genes and determining their mode of action (Martin *et al*, 1993).

In this review, we will summarize the main types of molecular markers that can be used for mapping genes and strategies for mapping monogenic or polygenic disease resistance will be presented.

## TYPES OF GENETIC MARKERS

Nuclear genetic markers differentiate genotypes and are inherited according to the Mendelian laws of inheritance. Through their linkage to important genes, markers facilitate the detection of differences in the genetic information carried by individuals.

### *Morphological markers*

Morphological markers generally correspond to qualitative traits that can be scored visually. They have been found in nature or as the result of mutagenesis experiments, for instance, in the tomato where more than 300 such mutants have been described (Tanksley and Mutschler, 1990). Morphological markers are usually dominant or recessive. Markers of this type have been used, for instance, the tomato *Tm-2* gene for resistance to tobacco mosaic virus (TMV) is linked to an anthocyaninless seedling marker (Robinson *et al*, 1970) and a peach mildew resistance gene is linked to the size of foliar glands (Connors, 1922).

### *Molecular markers*

Molecular markers reveal polymorphism at the protein or DNA level. They include biochemical markers, DNA restriction fragment length poly-

morphism (RFLP) (Beckmann and Soller, 1983; Tanksley, 1983) and markers obtained after amplification of a DNA sequence by the polymerase chain reaction (PCR) (Mullis and Faloona, 1987).

## Biochemical markers

Biochemical markers are proteins produced by gene expression. These proteins can be isolated and identified by electrophoresis and staining. Isozymes are proteins that catalyze the same enzymatic reaction; they are revealed on electrophoregrams through a colored reaction associated with the enzymatic activity. They are the product of the various alleles of one or several genes. Monomeric (fig 1a) and dimeric (fig 1b) isozymes are the most often used because the analysis of their segregation is easier. Isozymes are generally codominant. Examples of isozyme systems routinely used in plant breeding are: isozyme *Aps-1* for its linkage to the tomato resistance to nematode (*Meloidogyne* spp) from *Lycopersicon peruvianum* (Rick and Fobes, 1974); isozyme *Got2* for its linkage to the tomato resistance to *Fusarium oxysporum* race 3 from *L penellii* (Bournival *et al*, 1989); isozyme *Est5* for its linkage to the wheat resistance to *Meloigogyne naasi* from *Aegilops variabilis* (Yu, 1991); and isozyme *EpD1* for its linkage to the wheat resistance to *Cercosporella herpotrichoides* from *A ventricosa* (Doussinault, personal communication). It should be noted that, in most cases, the polymorphism of isozyme markers is rather poor within a cultivated species (*eg,* tomato). This explains why most of the known cases originate from interspecific crosses in which the resistance gene(s) come(s) from a wild relative.

## RFLP markers

DNA RFLP is the consequence of the variation that exists in the distribution and presence (or absence) of restriction sites recognized by an endonuclease. Endonucleases recognize short (3–9 bases) sequences and, once fixed on DNA at their site, cut DNA at a fixed point from the site. A single base change in the sequence is sufficient for the enzyme to fail to recognize the sequence, and consequently to fail to cut DNA at this site. Consider 3 sites A, B and C, in which all 3 are cut on double-stranded DNA and a base change prevents cutting at B on the other double-stranded DNA. Digestion of this heterozygous individual will yield fragments AB, BC from one

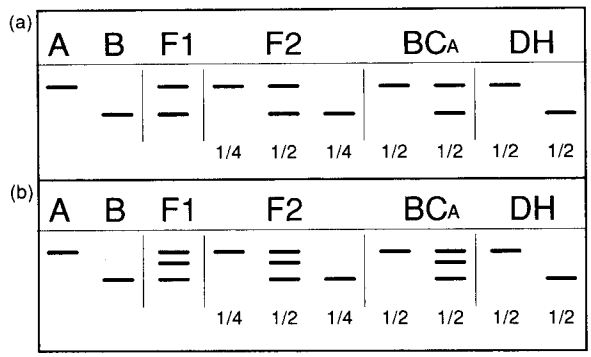


**Fig 1.** Example of segregation patterns and theoretical ratios (a) with a RFLP marker, with a specific PCR marker or with a codominant marker of a monomeric enzymatic system and (b) with a codominant marker of a dimeric enzymatic system according to the studied progenies issued from the cross between the parents A and B: F2, BC by the parent A and double haploids (DH).

double-stranded DNA and only AC from the other. Electrophoresis will reveal the difference in length between AB, BC and AC after hybridization with a probe, thus highlighting an RFLP that can be subjected to a linkage analysis. RFLP is thus generated whenever there is a loss or a creation of a restriction site by base substitutions, insertions, deletions, or chromosomal rearrangements. Such polymorphism can be detected by hybridization of a labelled probe to digested total DNA fragments separated by electrophoresis. Probes are preferably chosen as single copy genomic sequences or sequences with a low copy number. These markers are generally codominant (fig 1a).

## PCR markers

The polymerase chain reaction (PCR) uses the property of DNA to *melt* or *denaturate* when heated in solution (*ie* the 2 strands separate). This provides the opportunity for copying each strand through the operation of a polymerase adding nucleotides to form the complementary strand starting from sites at which *primers* have recognized complementary sequences in the template DNA. A thermocycler is used to vary the temperature in the reaction tubes according to optimal specified cycles. Primers can be randomly synthesized sequences (typically decamers) or sequences determined from a known sequence. Each cycle doubles the quantity of DNA copied from the small amount present at the start, thus exponentially amplifying the DNA sequence located between the 2 known DNA primers by copying it many times (20–45 cycles of amplifica-

tion) leading to a sufficient quantity for direct detection after electrophoresis. Hybridization of the PCR primers to template DNA occurs when the primers find complementary sequences on the template DNA at a convenient distance (< 2–3 kb). RAPD (random amplified polymorphic DNA) markers or specific PCR markers can be obtained depending on the type of primers and the annealing temperature used.

*RAPD markers*

RAPD markers were first described by Williams *et al* (1990) and Welsh and McClelland (1990). They are revealed using a single type of arbitrary 10-base primer and an annealing temperature of about 35°C. These markers cover the entire genome (single sequences to repeated sequences). One RAPD primer generally allows the amplification of several bands corresponding to several loci, which are generally dominant (presence against absence of the band). With RAPD markers, heterozygous individuals cannot be differentiated from homozygous dominant individuals. In addition, the progeny of backcrosses with the dominant parent does not segregate. Segregation patterns of RAPD markers are illustrated in figure 2.

*Specific PCR markers*

Pairs of specific primers 18- to 24-base long are used with an annealing temperature between 50 and 70°C to reveal loci. Such markers may be codominant if they reveal length polymorphism. The observed segregations will be the same as those described in figure 1a.

Specific PCR markers can be derived from primers, which match the nucleotide sequence of the ends of a DNA fragment, *eg,* an RFLP probe (sequence tagged sites or STS) or an expressed sequence tag (EST) (Höfte *et al*, 1993). When the amplified fragment does not show length polymorphism, a restriction enzyme is used on the amplified fragment and the resulting fragments are called CAPS (cleaved amplified polymorphic region) (Konieczny and Ausubel, 1993) (fig 3). Specific PCR markers can also be simple sequence repeats (SSRs) or microsatellites which are sequences of 1, 2, or 3 bases repeated more than 10 times and bordered with unique DNA sequences. Unique flanking primers are designed from the border sequences of the repeated region and allow the amplification of a specific microsatellite. The polymorphism revealed is due to modifications in the number of repeats (Beckmann and Soller, 1990; Hearne *et al*, 1992) and is of the codominant type. Finally, specific PCR markers can be sequence characterized amplified regions (SCAR markers) derived from RAPD fragments from which the ends have been sequenced and used as specific primers for amplification (Paran and Michelmore, 1993). The polymorphism is directly detected in the case of length polymorphism or after enzymatic restriction.

## *Comparative value of markers*

The comparative value of markers is described in table I. The number of available morphological and isozyme markers is generally small, except in tomato or maize. For instance, about 20 isozymes, corresponding to coding regions of the genome, are detected in most crops. Moreover, some isozyme markers dependent on the developmental stage of the plant must be assayed in specific tissues, and their expression may be conditioned by environmental conditions. DNA markers are not influenced by development or environment and generally have no effect on the phenotype. The genotype of a plant for the DNA markers can be determined at a very early developmental stage as soon as enough sample material has become available for DNA extraction. The number of observable markers is theoretically infinite. In practice, however, the number of markers is limited by the level of DNA polymor-

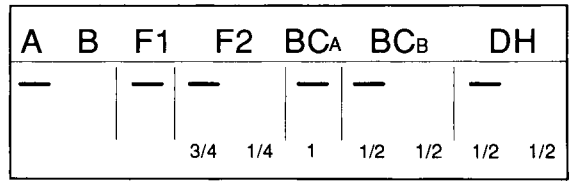


**Fig 2.** Example of a segregation pattern and theoretical ratios with a RAPD dominant marker according to studied progenies issued from the cross between the parents A and B: F2, BC by the parent A; and BC by the parent B and DH.

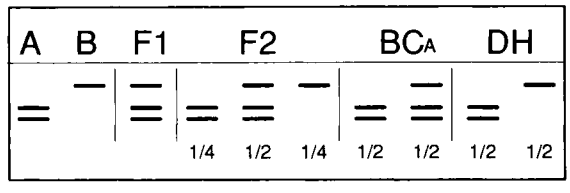


**Fig 3.** Example of a segregation pattern and theoretical ratios with a codominant specific-PCR marker digested with a restriction enzyme (CAPS) according to studied progenies issued from the cross between the parents A and B: F2, BC by the parent A and DH.

phism that exists between the genomes of the plants. DNA markers are considered to be a class of highly informative genetic markers, particularly microsatellites.

The selection of the appropriate markers for a study depends on the level of polymorphism in the plant species in the particular crosses. Although they are less time-consuming than RFLP markers, RAPD markers have been estimated to be more expensive when large numbers of individuals and markers need to be examined (Ragot and Hoisington, 1993).

## STRATEGIES FOR DEVELOPING MARKERS FOR DISEASE RESISTANCE GENES

The methods described here have the advantage of not requiring the knowledge of the gene product or expression. Identification of markers linked to disease resistance genes combines the use of detailed genetic maps and targeted mapping strategies.

### *Genetic linkage maps*

A genetic linkage map is a graphical representation of an array of loci. Linkage maps are developed following the analysis of a large number of markers in segregating progenies of polymorphic parents. Recombination rates are used to estimate the distance between the markers. There is no precise relationship between genetic distance expressed in centimorgans and physical distance expressed in base pairs. Segregating progenies, including F2 progeny (F2), backcross progeny (BC), doubled haploid lines (DH), and recombinant inbred lines (RIL), are used to study recombination between markers (Allard, 1956; Lefort-Buson *et al*, 1990). Selecting the plant material depends on the biology of the species and the objectives of the study. Detailed molecular maps have been developed for several crops, including tomato (Tanksley *et al*, 1992), maize (Gardiner *et al*, 1993), and rice (Causse *et al*, 1994).

As mentioned earlier, classical disease resistance studies provide information on the pattern of inheritance and may be useful to select a marker-based strategy. To simplify, we will consider that resistance is either qualitatively or quantitatively expressed. The use of DNA markers to study disease resistance in plants however has been focussed primarily on single locus resistance genes.

### Monogenic disease resistance

Marker-assisted selection is based on the concept that it is possible to infer the presence of a gene from the presence of a marker if a narrow linkage has been established between them. The expected proportions (probabilities) of each genotypic class for 2 independent or totally linked loci, in the F2, backcross, and doubled haploid progenies are summarized in table II. The likelihood of detecting a marker linked to a disease resistance gene is inversely proportional to the genetic distance between the marker and the gene. For a better estimate, the genetic distance between the marker and the gene must be calculated from a large population or better from several crosses. Genetic distances may greatly vary between crosses (Paterson *et al*, 1988; Messeguer *et al*, 1991).

Linkages have been frequently observed between markers and monogenic disease resis-

**Table I.** Comparative value of genetic markers.

| *Type* | *Number* | *Determinism* | *Environmental effects* | *Locus-specific* | *Feasibility* | *Cost* |
|---|---|---|---|---|---|---|
| Morphological markers | Few (simultaneously) | Dominant | Yes | Yes | ++++ | Growing plants |
| Isozymes | < 50 | Codominant | Yes/no * | Yes | +++ | + |
| RFLP | ~Unlimited | Codominant | No | Yes | + | +++ |
| RAPD | ~Unlimited | Dominant | No | No | +++ | +++(+) |
| STS/EST/CAPS/SCAR | ~Unlimited | Codominant | No | Yes | ++ | ++++ |
| Microsatellites/SSR | ~Unlimited | Codominant | No | Yes | ++ | ++++ |

* Depending on the isozyme.

tance by mapping on a genetic linkage map. Some examples are reported in table III. Isozymes, RFLP or RAPD are the most widely used markers for this strategy.

## Polygenic disease resistance

Complex disease resistances (*ie* quantitatively expressed) are generally assumed to be under oligogenic or polygenic control (Mather and Jinks, 1971) and/or influenced by the environment. Quantitative trait loci, or QTLs (Geldermann, 1975), are considered to identify chromosome sites at which genes that have effect on quantitative traits can be located. The quantitative nature of a resistance trait would result from the simultaneous but independent allelic variation of the genes involved and the effect of the environment (Yule, 1906; East, 1915). The search for linkages between molecular markers and QTLs is based on this hypothesis.

### *Detection of QTLs*

The detection of linkages between markers and QTLs can be performed using various statistical methods. The statistical approach using the analysis of variance estimates the degree of association between a genotypic marker (an allelic form) and a phenotypic trait. Phenotypical values are the dependent variables and the genotypic markers correspond to the treatment or the factor (source of variation). Analysis of variance models of increasing complexity provide information on the genetic basis of the resistance, for instance, the effect of individual markers (one-way Anova), the effect of pairs of markers (epistasis by two-way Anova) (Lefebvre, 1993).

The interval mapping approach (Lander and Botstein, 1989) considers linkages between markers. Using the maximum likelihood equation, the method provides an estimate, expressed as LOD score, of the likelihood of the presence of a QTL for regular intervals throughout the genome based on flanking marker information. The LOD scores actually depend not only on the localization of the QTL with respect to the flanking markers and on the magnitude of its effect, but also on the probability that there is a QTL there. While this should be kept in mind when examining the curves representing LOD, this method is very powerful, because it accounts for recombination rates between markers. To use this method therefore requires the markers to have been mapped and the trait to have a Gaussian distribution, a condition which is not always satisfied in the study of disease resistance genes (semi-quantitative data).

Analysis of variance and interval mapping are the most currently used methods. Since disease resistance is often assessed with ordinal scales and data do not always show a normal distribution, researchers have been testing putative QTLs with non-parametric statistical tests (Kreike *et al*, 1993; van Ooijen *et al*, 1993; Young *et al*, 1993). Other methods, using maximum likelihood, mean squares, linear and multiple regressions, have been described (Knapp, 1991; Carbonell *et al*, 1992; Haley and Knott, 1992; Rodolphe and Lefort, 1993).

**Table II.** Expected proportions of genotypical classes for 2 biallelic linked or independent loci in F2, BC and DH progenies.

| | *Totally linked loci* | | | | | *Independent loci* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *R M1* / *R M1* | *R M1* / *r m1* | *r m1* / *r m1* | *R M2* / • *M2* | *r M2* / *r M2* | *R M2* / • *m2* | *r M2* / *r m2* | *R m2* / • *m2* | *r m2* / *r m2* |
| F2 | 1/4 | 1/2 | 1/4 | 3/16 | 1/16 | 6/16 | 2/16 | 3/16 | 1/16 |
| BCA | 1/2 | 1/2 | | 1/2 | | 1/2 | | | |
| BCB | | 1/2 | 1/2 | | | 1/4 | 1/4 | 1/4 | 1/4 |
| DH | 1/2 | | 1/2 | 1/4 | 1/4 | | | 1/4 | 1/4 |

M1-m1 is linked to the R-r locus; M2-m2 is not. Marker loci (M1-m1 and M2-m2) are codominant. The R allele conferring the resistance is dominant. Parental genotypes: RM1/RM1 or R/R M2/M2 for the parent A, and rm1/rm1 or r/r m2/m2 for the parent B. BCA = (F1 x parent A) and BCB = (F1 x parent B).

**Table III.** Examples of marker identification for resistance genes using mapping.

| *Host species* | *Pathogen* | *Resistance gene* | *Reference* |
|---|---|---|---|
| Tomato | Nematodes | *Mi* | Rick and Fobes, 1974<br>Messeguer *et al*, 1991<br>Klein-Lankhorst *et al*, 1991 |
| | *Fusarium oxysporum* sp *lycopersici* race 1 | *I1* | Sarfatti *et al*, 1991 |
| | *Stemphyllium* spp | *Sm* | Behare *et al*, 1991 |
| Potato | Nematodes | *Gro1*, *H1* | Barone *et al*, 1990<br>Gebhardt *et al*, 1993 |
| | Potato virus X | *Rx1* | Ritter *et al*, 1991 |
| Pepper | Tobacco mosaic virus | *L* | Lefebvre *et al*, 1994 |
| Lettuce | *Bremia lactucae* | Several *Dm* genes | Landry *et al*, 1987 |
| Pea | Pea seed-borne mosaic virus | *sbm-1* | Timmerman *et al*, 1993 |
| | *Fusarium oxysporum* f sp *pisi* race 1 | *Fw* | Dirlewanger *et al*, 1994 |
| | *Erysiphe polygoni* | *er* | Dirlewanger *et al*, 1994 |
| | Pea common mosaic virus | *mo* | Dirlewanger *et al*, 1994 |
| Common bean | *Colletotrichum lindemuthianum* | *Are*, *RVI* | Adam-Blondon *et al*, 1994a |
| Mungbean | *Callosobruchus* (Bruchids) | | Young *et al*, 1992 |
| Rice | *Orseolia oryzae* biotype 1 (Dipteran) | *Gm2* | Mohan *et al*, 1994 |
| Barley | *Erysiphe graminis* f sp *hordei* | *MI-o* | Hinze *et al*, 1991 |
| | Barley yellow mosaic virus and Barley mild mosaic virus | *ym4* | Graner and Bauer, 1993 |
| Maize | Maize dwarf mosaic virus | | McMullen and Louie, 1989 |
| | Wheat streak mosaic virus | | McMullen and Louie, 1991 |

With molecular markers, polygenic disease resistance can be partitioned and individual effects can be examined (components of resistance). Results of genetic studies of complex interactions have been reported, including first insect resistance in tomato (Nienhuis *et al*, 1987), and then quantitative resistance to pathogenic fungi and bacteria, and to nematodes (see references in the following). In addition, QTL mapping could be useful for identifying loci involved in quantitative components of resistance to viral infections such as rate of multiplication, movement in the host and disease progression. New genes for partial resistance might be identified by this approach.

*Number and effects of QTLs*

Depending on the host–parasite interaction considered, from 2 (Heun, 1992; Landry *et al*, 1992; Bubeck *et al*, 1993; Freymark *et al*, 1993; Kreike *et al*, 1993; Concibido *et al*, 1994) to 7 (Schön *et al*, 1993) QTLs have been identified to explain partial disease resistance traits, confirming that these traits were under polygenic control. QTLs were generally found to be distributed across several linkage groups.

The total phenotypic variation accounted for by QTLs is variable. In the potato cyst nematode interaction, 2 QTLs explained 14% of the phenotypic variation (Kreike *et al*, 1993) and 4 QTLs accounted for 75% of the variation of the resistance of bean to common bacterial blight (Nodari *et al*, 1993). One QTL was found to have major effects on the resistance of pea plants to *Ascochyta* blight (Dirlewanger *et al*, 1994) and on the resistance of pepper to *Phytophthora* root rot (Lefebvre, 1993).

Analyses have generally been conducted using F2 and F3 progenies, which allows the detection of dominance in the action of genes located at QTLs. No general rules seem to apply. While some loci have additive effects, others have partially or completely dominant ones. One case of overdominance was reported for a QTL affecting the interaction between bean and *Rhizobium* (Nodari *et al*, 1993).

Several authors have pointed out that the susceptible parent can contribute to resistance since some QTLs have been shown to have effect contrary to parental behavior (Bubeck *et al*, 1993; Figdore *et al*, 1993; Freymark *et al*, 1993; Lefebvre, 1993; Nodari *et al*, 1993; Schön *et al*, 1993; Young *et al*, 1993; Dirlewanger *et al*, 1994). The recombination of susceptible and resistant alleles originating from both parents may explain the occurrence of either resistant or susceptible transgressive segregants in progenies of 'resistant x susceptible' crosses.

Gene mapping in different populations and environments allows the determination of the effect of the genetic background and genotype x environment effects, and the detection of specific genetic factors originating from diverse sources of resistant germplasm. For instance, European corn borer resistance in maize appears to be more consistent across locations (Schön *et al*, 1993) than gray leaf spot resistance (Bubeck *et al*, 1993). Two sources of resistance to gray leaf spot resulted from different QTLs providing an opportunity for multiple resistance (Bubeck *et al*, 1993). To differentiate QTLs from 2 cultivars of barley with quantitative resistance to powdery mildew, Heun (1992) used a segregating DH population derived from a cross between the cultivars. It remains to check that the 2 resistances behave as additive.

### *Epistasis*

A 2-factor analysis of variance can detect interactions between the genes associated with markers. This has rarely been tested. A few authors (Bubeck *et al*, 1993; Kreike *et al*, 1993; Concibido *et al*, 1994) tested the interaction between 2 markers associated with additive effects at QTLs but found no significant interaction, which indicated that the loci were additive and that epistasis between the QTLs was not important. In the common bean, the frequency of significant interactions for the number of *Rhizobium* nodules was similar to the frequency of type I error suggesting that the interactions could represent false positives (Nodari *et al*, 1993). A significant digenic epistasis ($P < 0.001$) was detected for 1 couple of markers associated with resistance against second-generation European corn borer (Schön *et al*, 1993). Nienhuis *et al* (1987) also suspected additive x additive epistatic interactions among 'additive QTLs'. In these studies, QTLs with exclusively epistatic but no additive or dominance effects could not be detected. Only Nodari *et al* (1993) tested all possible interactions of markers for the resistance to common bacterial blight in bean. Again a small proportion of interactions were significant with a threshold level of 0.05, suggesting that interactions were not important for this trait. In our model of the resistance of pepper to *Phytophthora* root rot (Lefebvre *et al*, in preparation), we detected several significant digenic interactions ($P < 0.0005$) that can explain up to 30% of the phenotypic variation. Epistasis effects are certainly partly responsible for the difficulties of transferring polygenic resistances into improved varieties. Interactions can also occur between alleles from the resistant parent and the susceptible parent. This suggests that epistatic effects probably also contribute to the emergence of transgressive lines.

### *Components of resistance*

Studies of the components of a host–pathogen interaction, for instance, epidemiological characters (receptivity of the plant, inducibility of the resistance, lesion number per leaf, lesion size, incubation period, symptom development, latent period, number of propagules per unit area and disease severity), have revealed QTLs for specific steps of the interaction or QTLs common to several steps. For example, in mungbeans, a QTL appeared to be specifically associated with a delay in the development of the resistance to powdery mildew (Young *et al*, 1993). The resistance of maize to *Exserohilum turcicum* appeared to be controlled by 3 QTLs affecting both lesion number and disease severity, 1 QTL affecting both lesion size and disease severity, 1 QTL specific to lesion size, and 1 QTL specific to

disease severity (Freymark *et al*, 1993). The resistance of pepper to *Phytophthora* root rot (Lefebvre *et al*, in preparation) is determined by QTLs specifically affecting 1 resistance criterion corresponding to a component of the resistance or to a particular organ inoculated as well as by QTLs affecting several resistance criteria. To date, we do not know whether QTLs common to several traits result from a pleiotropic effect of a single gene or correspond to a cluster of resistance genes.

### *Strategies for targeted mapping*

It is possible to identify markers for disease resistance genes without drawing a genetic linkage map, which is a time-consuming procedure. The direct use of markers is essentially limited to monogenic traits since it consists of identifying a particular genomic region coding for the trait. Examples (see references in the following) include studies using aneuploid lines to identify the chromosomes or chromosome arms that carry disease resistance genes, and near isogenic lines or bulk segregant analysis to identify markers located near disease resistance genes.

#### Aneuploid lines

Aneuploid lines are quite useful to analyze individual chromosomes. The lines may be obtained by repeated backcrossing of an interspecific hybrid with a recurrent parent. After selfing, the progeny carries the chromosomes of the recurrent parent and an additional chromosome, or pair of chromosomes, from the other parent, and the loci differentiating the 2 species are located on the additional chromosome(s). The markers are not ordered on the resulting chromosome map (*eg*, Chèvre *et al*, 1991).

Using aneuploid lines, it is possible to identify the chromosome(s) carrying resistance gene(s). Examples in the literature include nematode resistance in relatives of sugar beet (Jung *et al*, 1986) and wheat (Yu, 1991), and blackleg resistance in oilseed rape from related species carrying B chromosomes (Zhu *et al*, 1993; Chèvre *et al*, in preparation).

When the genes are located on different chromosomes, aneuploid lines can be used to identify the individual effects of genes or to analyze resistance that is expressed at different developmental stages (Chèvre *et al*, in preparation). Moreover, when related species are resistant to the same pathogen and their chromosomes are added to a susceptible genotype, it is possible, by comparison of their aneuploid lines, to determine whether a syntenic group is involved (Jung *et al*, 1986).

Markers for disease resistance genes on additional chromosomes can also be used to characterize translocations or recombinant resistant lines carrying extremely small introgressed chromosomal segments.

#### Near-isogenic lines

Near-isogenic lines differ by one or a small number of loci. They are produced by repeated backcrossing of an F1 hybrid to the susceptible parent. RFLP or RAPD analysis of these lines is a powerful tool to map resistance genes against viruses, fungi, and nematodes (table IV).

The production of near-isogenic lines is, however, time-consuming since a minimum of 6 backcrosses are required to ensure that the genomes are mostly identical except for the small target segment around the specific gene. Researchers have compared several pairs of near isogenic lines to reduce the probability of detecting false positives in regions unlinked to the target segment. Addition lines can also be used to confirm the validity of the markers. For instance, to confirm the introgression of the *Mi* gene from *L pennelli* into tomato, Klein-Lankhorst *et al* (1991) compared pairs of near-isogenic lines with a resistant tomato chromosome 6 substitution line.

The limiting factor with the method is the polymorphism rate between the lines. Markers are frequently obtained when a resistance gene is introgressed from a related species. In this case, recombinations are known to be suppressed in genotypes heterozygous for a foreign chromosomal segment, so the size of an introgressed segment is likely to be larger in terms of DNA base pairs than the size estimated from the genetic linkage map (Klein-Lankhorst *et al*, 1991).

With molecular markers, it is possible to introduce Mendelian loci and to determine their relative contribution to partial resistance (Yu *et al*, 1991). The study of segregating populations remains a requirement to confirm that the markers are tightly linked to the disease resistance gene and to define their distance and their order.

#### Bulk segregant analysis

This method has been developed by Michelmore *et al* (1991) to map *Dm* genes of *Lactuca sativa*

conferring resistance to *Bremia lactucae*. With this approach, DNA samples from susceptible or resistant plants from a segregating population are bulked separately. The comparison of the bulks using RAPD or RFLP markers allows the identification of markers linked to the gene of interest. The target region is then tested against a random genetic background. The pattern obtained for an F2 population is illustrated in figure 4.

Any segregating population (F2, BC, HD, *etc*) can be studied with this method. The efficiency of the analysis depends first on the polymorphism of the parents for the target region and on the size of the bulk.

The size of the bulk is based on the frequency with which unlinked loci can be detected between the bulk and on the maximum required distance between the marker and the gene. For example,

**Table IV.** Examples of marker identification for resistance genes using near-isogenic lines.

| *Host species* | *Pathogen* | *Resistance gene* | *Reference* |
|---|---|---|---|
| Tomato | Tobacco mosaic virus | *Tm-2a* | Young *et al*, 1988<br>Young and Tanksley, 1989 |
| | Tobacco mosaic virus | *Tm-1* | Levesque *et al*, 1990 |
| | *Fusarium oxysporum* f sp *lycopersici* race 2 | *I2* | Sarfatti *et al*, 1989 |
| | *Clasdosporium fulvum* | *Cf-9* | van der Beek *et al*, 1992 |
| | *Pseudomonas syringae* | *Pto* | Martin *et al*, 1991 |
| | *Meloidogyne* spp | *Mi* | Klein-Lankhorst *et al*, 1991 |
| Potato | Potato virus X | *Rx2* | Ritter *et al*, 1991 |
| Lettuce | *Bremia lactucae* | *Dm1*, *Dm3*, *Dm11*, *Dm15*, *Dm16* | Paran *et al*, 1991 |
| Common bean | *Uromyces appendiculatus* var *appendiculatus* | *Up2* | Miklas *et al*, 1993 |
| | *Collethotrichum lindemuthianum* | *Are* | Adam-Blondon *et al*, 1994b |
| Soybean | *Phytophthora megaspersma* f sp *glycinea* | *Rps1*, *Rps2*, *Rps3*, *Rps4*, *Rps5* | Diers *et al*, 1992 |
| Rice | *Pyricularia orizae* | *Pi-2(t)*, *Pi-4(t)* | Yu *et al*, 1991 |
| Barley | *Erisyphe graminis* f sp *hordei* | *Mla* | Schüller *et al*, 1992<br>Jahoor *et al*, 1993 |
| | *Erisyphe graminis* f sp *hordei* | *ml-o* | Hinze *et al*, 1991 |
| | *Rhynchosporium segalis* | *Rh* | Barua *et al*, 1993 |
| Wheat | *Erisyphe graminis* f sp *tritici* | *Pm3* | Hartl *et al*, 1993 |
| | *Helminthosporium maydis* | *rhm* | Zaitlin *et al*, 1993 |
| Oat | *Puccinia graminis* and *P coronata* | *Pg3* | Penner *et al*, 1993 |
| Maize | *Helminthosporium turcicum* | *Ht1* | Bentolila *et al*, 1991 |

for a bulk composed of 10 plants, the frequency of false positives will vary from 2 x $10^{-6}$ for an F2 population to 2 x $10^{-3}$ for DH or BC1 plants. The higher the number of plants in the bulk, the lower the frequency of false positives. If the number of plants is small, the frequency of markers unlinked to the resistance gene may increase but a greater number of markers can be found. In any case, the linkage must be confirmed by the analysis of the segregating population, which will identify the false positives. The degree of linkage is finally determined according to the analysis of the segregating population. When the population is homozygous for most loci, fewer plants per bulk can be used. This was illustrated by the mapping of the *Up2* gene of common bean conferring resistance to *Uromyces appendiculatus* with a bulk of 3 plants from a BC6F2 population (Miklas *et al*, 1993). The linked RAPD marker can be used to combine the *Up2* gene with other rust resistance genes (Haley *et al*, 1993). In studies with doubled haploids, 2 strategies have been proposed: (i) to bulk more than 10 plants (as proposed by Pineda *et al*, 1993, who obtained RFLP markers linked with gene *H1* of *Solanum tuberosum* conferring resistance to *Globodera rostochiensis*); or (ii) to analyse simultaneously NILs and bulks made of 5 doubled haploids or bulks of NILs (as proposed by Barua *et al*, 1993, who mapped the *Rh* locus conferring resistance to *Rhynchosporium secalis* in barley using RAPD markers, and Haley *et al*, 1994, who mapped the *Ur-3* locus conferring the resistance to *Uromyces appendiculatus* in common bean also using RAPD markers).

RAPD markers appear to be more efficient than RFLP markers with the bulk segregant analysis method. Individual primers can be used to detect multiple loci with a random distribution. The sensitivity of the PCR technology is such that one recombinant in a bulk of 10 plants can be detected (Michelmore *et al*, 1991). SCAR markers are derived from RAPD markers in order to increase the repeatability and reliability of the latter (Paran and Michelmore, 1993).

Regardless of the marker used, polymorphism is not detected more than about 30 cM from the target region. Bulk segregant analysis is a very efficient technique to saturate a region with molecular markers.

## DISCUSSION AND CONCLUSIONS

### *Perspectives opened by molecular mapping of resistance in plants*

The understanding of the mechanisms underlying monogenic and polygenic disease resistances is steadily progressing. In some cases, markers linked to disease resistance loci can now be used for marker-assisted selection (MAS) programs, eliminating the need for traditional disease testing procedures. Melchinger (1990) optimized a design for retaining the minimum number of individuals in each generation, relying on the recombination rate between the target gene and 1 or 2 markers. For a major resistance gene, marker-based recurrent backcross programs are frequently used (Young and Tanksley, 1989). MAS can be extremely useful to cumulate several resistance genes ('pyramiding' resistance genes). A future application of MAS will be in quantitative resistance loci. When a small number of QTLs are involved, the technique becomes similar to that used to select qualitative traits. The more loci there are segregating for a trait, the larger the number of individuals which must be characterized to have a high probability of recovering the favorable set of marker alleles at all the interesting loci. If the trait is controlled by a large number of QTLs with small effects, the probability of identifying markers linked to all the QTL is low and moreover there is a high probability of finding a single false QTL representing the resultant effect of many small effect genes dispersed on a chromosome arm (Gallais and Rives, 1993), making selection difficult. A selection index including both molecular marker information and

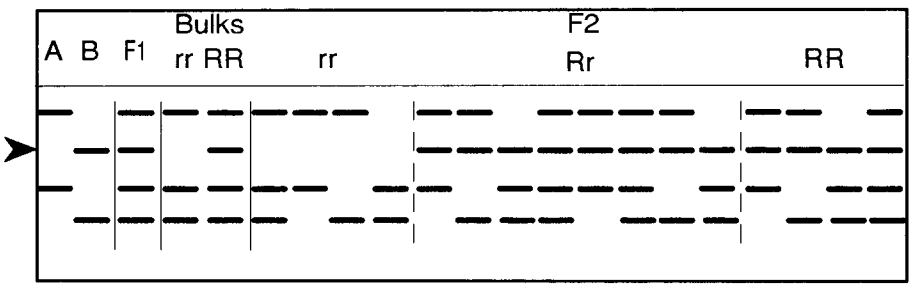


**Fig 4.** Example of a pattern of bulks derived from F2 individuals homozygous for resistance (RR) or susceptibility (rr) and of the F2 individuals. The arrow shows the dominant locus linked to the allele conferring the resistance (Michelmore *et al*, 1991).

phenotypic scores will produce more progress from selection than marker selection or phenotypic selection alone unless heritability of the trait is 1 (Lande and Thompson, 1990). Dudley (1993) reviewed methods and results concerning MAS for traits controlled by a large number of QTLs and discussed methods of combining data from different markers and different traits. The potential of MAS in quantitative genetics remains unclear because it depends on the relative cost and the actual complexity of molecular biology techniques. There are still technical limitations for generalizing their use everywhere (DNA extraction, Southern transfer and hybridization for RFLP). With the development of the PCR-based techniques, MAS is greatly simplified.

### *Random or organized structure of resistance systems in plants?*

Several studies have revealed overlapping or tight linkage between resistance loci with known qualitative effects and QTLs affecting quantitative resistance. These results re-open the debate about van der Planck's concepts (1968) of horizontal and vertical resistance (Freymark *et al*, 1993). Nelson (1978) proposed that quantitative resistance may be the accumulation of residual effects of qualitative resistance genes that are no longer effective. More generally, Robertson (1989) suggested that genes with quantitative effects should be allelic to genes with qualitative effects. Support for this hypothesis is provided by studies of plant height in maize (Beavis *et al*, 1991). It is also provided by studies of Freymark *et al* (1993), who determined that loci in the vicinity of 3 monogenic resistance genes to *Exserohilum turcicum* had minor effects on 2 quantitative resistance components to this pathogen. In contrast, Heun (1992) and Kreike *et al* (1993) observed no residual effects of monogenic resistance loci on quantitative resistance to powdery mildew in barley (Heun, 1992) and to potato cyst nematode in potato (Kreike *et al*, 1993). In pea, a QTL linked to resistance to *Ascochyta pisi* was located near a monogenic resistance locus to powdery mildew (Dirlewanger *et al*, 1994). Similarly, in pepper, we found a QTL affecting the resistance to *Phytophthora* root rot mapping in the vicinity of the monogenic resistance locus *L* to TMV (Lefebvre *et al*, unpublished). In the last 2 examples, QTLs displayed reverse effects relative to parental values. This is to be expected since the monogenic resistance and the polygenic resistance originated from different parents.

A genomic segment may be associated with several host–pathogen interactions (quantitative effects). The segment may contain multiple QTLs or a unique factor with pleiotropic effects. Such a region has been found for *Rhizobium* nodule number and resistance to common bacterial blight in common bean (Nodari *et al*, 1993). Similarly, the resistance to *Phytophthora* root rot and to potyviruses in pepper is influenced by common genomic regions (Caranta *et al*, personal communication). The results suggest that common defense mechanisms involved perhaps in 'general' resistance may operate in host–pathogen interactions. To check this hypothesis, markers corresponding to cloned disease resistance genes with a known function could be mapped and their localization compared with that of QTLs for resistance genes.

The examples cited above indicate that certain genomic regions play an important role in disease resistance. These regions may correspond to either an allelic series at a given resistance locus or a number of tightly linked loci. This may also mean that a same recognition or delivery signals may be shared by several pathogens. In addition, mechanisms to generate gene complexes probably exist. Hulbert and Bennetzen (1991) observed a very high rate of meiotic instability for the *Rp1* genes. The instability resulted from unequal cross-over probably due to 'slippage'. Such recombinational events may be responsible for the expansion or contraction of disease resistance loci. Recombination 'hot spots' may be analogous to that observed for the human major histocompatibility complex, a genomic region encoding the mammalian immune system (Dangl, 1992). The existence of clusters of resistance genes offers an opportunity to quickly reveal markers closely linked to a new resistance gene in analyzing first the markers known to be linked to clusters of resistance genes (Maisonneuve *et al*, 1994).

Synteny studies (Tanksley *et al*, 1992; Prince *et al*, 1993) have made it possible to compare genomic regions involved in disease resistance in related species. For instance, a QTL for resistance to the potato cyst nematode has been mapped on potato chromosome 11, while the tomato resistance loci *Sm* and *I2* are located on a tomato chromosome homeologous to potato chromosome 11 (Sarfatti *et al*, 1989; Behare *et al*, 1991; Kreike *et al*, 1993). Similarly, the *L* locus for resistance to TMV in pepper is linked to

an RFLP marker on tomato chromosome 11 (Lefebvre *et al*, 1994). The *Gro1* resistance locus in potato (Barone *et al*, 1990) is closely linked to markers for the *I1* resistance locus on tomato chromosome 7 (Sarfatti *et al*, 1991). The *H1*, *R1* and *Rx2* resistance loci have been mapped on potato chromosome 5 (Ritter *et al*, 1991; Leonards-Schippers *et al*, 1992; Gebhardt *et al*, 1993; Pineda *et al*, 1993) and the tomato *Pto* resistance locus also maps on the homeologous tomato chromosome 5 (Martin *et al*, 1993). In pepper, 1 marker located on the tomato chromosome 5 is linked on the pepper map to a QTL influencing the resistance to *Phytophthora* root rot (Lefebvre, 1993).

Markers linked to disease resistance genes may be useful for cloning and sequencing the genes and to investigate their function. Five resistance genes have been cloned: the maize *Hm1* gene (Johal and Briggs, 1992); the tomato *Pto* gene (Martin *et al*, 1993); the tomato *Cf-9* gene (Jones *et al*, 1994), the arabidopsis *Rps2* gene (Bent *et al*, 1994; Mindrinos *et al*, 1994); and the tobacco *N* gene (Whitham *et al*, 1994). The last 4 correspond to the gene-for-gene model. The understanding of the function and regulation of the resistance genes and their interaction with the pathogen will be a key element in obtaining durable resistance.

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