CHAPTER 2

GENETICS OF DISEASE RESISTANCE

BASIC CONCEPTS AND APPLICATION IN RESISTANCE BREEDING

BEAT KELLER[1], CATHERINE FEUILLET[1] & MONIKA MESSMER[2]

[1] *Dept. of Plant Molecular Biology, Institute of Plant Biology University of Zürich, CH-8008 Zürich, Switzerland (bkeller@botinst.unizh.ch)*
[2] *Dept. of Quality and Resistance Breeding, Swiss Federal Research Station for Agroecology and Agriculture, Reckenholzstrasse 191 CH-8046 Zürich, Switzerland*

Summary

Disease resistant plants are one of the prerequisites for sustainable agriculture. To understand and rationally use the naturally occurring disease resistance, its genetic basis has been investigated in great detail. These studies showed that there are two different genetic mechanisms for disease resistance: monogenic resistance is based on single genes whereas quantitative resistance depends on two or more genes. In most cases, single resistance genes confer complete resistance but are only active against certain races of the pathogen, i.e. they show a genetic interaction with genes from the pathogen. This resistance is based on an active recognition event between the product of the plant resistance gene and the product of the avirulence gene of the pathogen. Resistance genes are clustered at some loci in the genome or exist as different alleles conferring resistance towards specific pathogen races. Quantitative resistance shows no obvious genetic interaction with the pathogen and slows down the disease development by increasing latency period and other parameters related to the epidemic. Resistance breeding in crop plants depends on both types of resistance. Monogenic resistances are easy to work with but are frequently not durable. Consequently, quantitative resistance is preferred. The application of molecular markers has allowed the genetics of quantitative resistance to be determined and quantitative trait loci involved in resistance to be identified. Molecular markers have also contributed to improved breeding strategies for monogenic resistance genes in order to combine them in the "gene pyramiding" strategy for a more durable resistance. Finally, molecular markers have allowed the isolation of the first disease resistance genes. The cloning of such genes from crop plants and their wild relatives will open new possibilities for their sustainable use in breeding.

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I. Introduction

The classical and molecular genetics of disease resistance in plants is one of the intellectually most challenging and practically important research topics in plant biology. The application of naturally occurring resistance in crop breeding has contributed greatly to the control of plant diseases. Recently, there has been renewed and increasing interest in genetic resistance for several reasons: in many developing countries poor farmers do not have the financial resources and the education for a safe application of pesticides whereas natural resistance is a potentially cheap and efficient way to fight diseases. It has also become clear that the use of pesticides can cause considerable environmental damage. Consequently, agricultural policies in many developing and industrialized countries have the goal to reduce the overall use of pesticides.

Originally, the genetic analysis of resistance grew out of the need to understand the basis of field observations: some plant lines were resistant to a particular disease whereas others were susceptible. In addition, resistant crops could become susceptible, even after showing good resistance in the field for several years. The genetic characterization of disease resistance in plants has been essential for the understanding of plant-pathogen interactions. It has allowed the formulation of some of the key concepts in plant pathology, thereby creating the framework for rational strategies to control plant diseases. These concepts have greatly and very successfully contributed to an efficient breeding for disease resistance in many crop plants. It is estimated that at least 75% of all important agricultural crops have an effective inherited resistance against at least one pathogen and 98% of all grain and forage crops have an inherited resistance component against one or more diseases (Schumann, 1991).

In this chapter, the basic concepts of disease resistance which resulted from genetic analysis will be described. Methodological and technical advances for the study of the genetics of resistance and their consequences for practical breeding will be discussed. Finally, we will describe in detail our current understanding of the genetics of quantitative resistance which is based on the action of several genes.

II. The Gene-For-Gene Hypothesis for the Description of Plant-Pathogen Interactions

It was discovered early in the century that in some cases disease resistance was inherited as a monogenic trait following the laws of classical Mendelian genetics. For example, Biffen (1905) demonstrated that the resistance against yellow (stripe) rust in the wheat variety "Rivet" was due to a single, recessive gene. Recessive resistance genes are actually rare and later the more frequent dominant or semidominant resistance genes were found. However, initially there was considerable confusion about the observations. Even with genetically stable (i.e. true breeding) wheat lines, the reaction to rust differed between different locations and years. Artificial infections with spores collected in the field resulted in inconsistent data. The explanation for this variability only became clear after the discovery and characterization of defined pathogen races. The differential

reaction of a variety with a resistance gene towards particular races of the pathogen suggested that there was genetic variability in the pathogen which specifically interacted with monogenic resistances in the plant. Based on reaction to the pathogen, Ausemus *et al.* (1946) described three dominant, monogenic resistances against the wheat leaf rust pathogen (*Puccinia recondita* f. sp. *tritici*) and five dominant genes against stem rust caused by *Puccinia graminis* Pers. f. sp. *tritici*. Additional studies with other pathosystems such as barley or wheat powdery mildew, flax rust, potato late blight and lettuce downy mildew revealed that dominant monogenic resistance traits in plants were quite common. Such resistance genes were the basis for Flor's pioneering analysis of resistance.

H.H. Flor was the first plant pathologist who analyzed the genetics of a resistance interaction simultaneously in both the plant and the pathogen. He studied the interaction between flax (*Linum usitatissimum*), a crop plant mainly used for fiber production, and the fungal disease flax rust caused by the Basidiomycete *Melampsora lini*. From these studies he formulated the so called gene-for-gene hypothesis as the most convincing explanation of the observed phenomena (Flor, 1942; 1955; 1971). Flor made his observations after infecting flax lines carrying different resistance genes with the progeny of crosses between different races of the rust pathogen. He used a cross of two different races of flax rust to develop segregating F_2 cultures. These cultures were then tested for the ability to multiply and grow on more than 30 different varieties of flax that had previously been selected as carrying single genes for rust reaction (Flor, 1955). The conclusion from these studies was that genetic factors of both the plant and the pathogen are required for a successful defense reaction of the plant. The specificity of a plant-pathogen interaction is determined by the interaction of an avirulence gene product encoded by a dominant gene in the pathogen and a product of the resistance gene from the plant. The basis of the plant resistance reaction is therefore a specific recognition between the two components. This recognition triggers further physiological defense reactions resulting in hypersensitive cell death and the accumulation of molecules which are toxic for the pathogen (see chapter 7; Lamb, 1994). This is also called an incompatible interaction between the plant and the pathogen. In the absence of either the resistance gene product or the avirulence gene product, there is no recognition of the pathogen by the plant. This allows the further growth of the pathogen, resulting in a compatible interaction and susceptibility. Thus, a mutation in either the avirulence or the resistance gene which results in a loss of function will result in a change from an incompatible to a compatible interaction. The presence of a resistance gene in the host plant therefore exerts a strong selective pressure for a mutation in the avirulence gene if the product of the avirulence gene is not essential for the survival of the pathogen. This selection pressure has important epidemiological consequences for the development of new pathogen races and the losses in crop production (see chapter 3 and below). Thus, resistance in the gene-for-gene interaction is race-specific whereas susceptibility is not specific. The genetic basis of specific resistance is best understood by the quadratic check (Fig. 1A) that can be used to describe the gene-for-gene interaction. In this graphical description, resistance occurs only when both a dominant *R* gene from the plant as well as the dominant avirulence gene *A* from the pathogen are present in the upper left quadrant. In all the other

quadrants the interaction is compatible, resulting in susceptibility. To prove a gene-for-gene interaction, the quadratic check must be reciprocal, i.e. it must be true for at least two resistance genes in the host and two matching avirulence genes in the pathogen (Fig. 1B) (Van der Planck, 1978). If this condition is fulfilled, a gene-for-gene interaction occurs in this particular disease. A more molecular model derived from the observation of the dominant character of both the avirulence gene and the resistance gene is shown in Fig. 2. The product of the resistance gene in this model would be a receptor that actively recognizes a direct or indirect product of the avirulence gene. Only the receptor-ligand interaction (Fig. 2A) results in specific recognition indicated by the hypersensitive response and disease resistance.

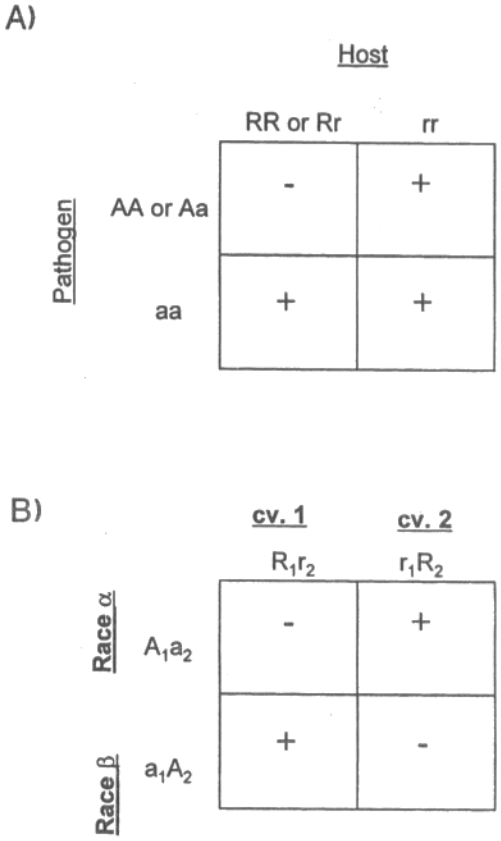

Figure 1. The gene-for-gene interaction. Quadratic check of gene combinations and the resulting different interaction types in a gene-for-gene interaction. The pathogen can grow in the compatible (+), but not in the incompatible (-) interactions. A indicates a dominant avirulence gene in the pathogen, R a dominant resistance gene in the plant.
(A): The quadratic check for a single locus in the host and in the pathogen. Only the combination of the dominant resistance and the dominant avirulence gene results in plant resistance in the upper left quadrant.
(B): Reciprocal check for two genetic loci of resistance (R_1 and R_2) in the two plant cultivars (cv. 1 and 2) and the corresponding two avirulence loci in two pathogen races (A_1 and A_2). The combination of R_1 and A_1 or R_2 and A_2 results in plant resistance. The reciprocal check defines a gene-for-gene interaction.

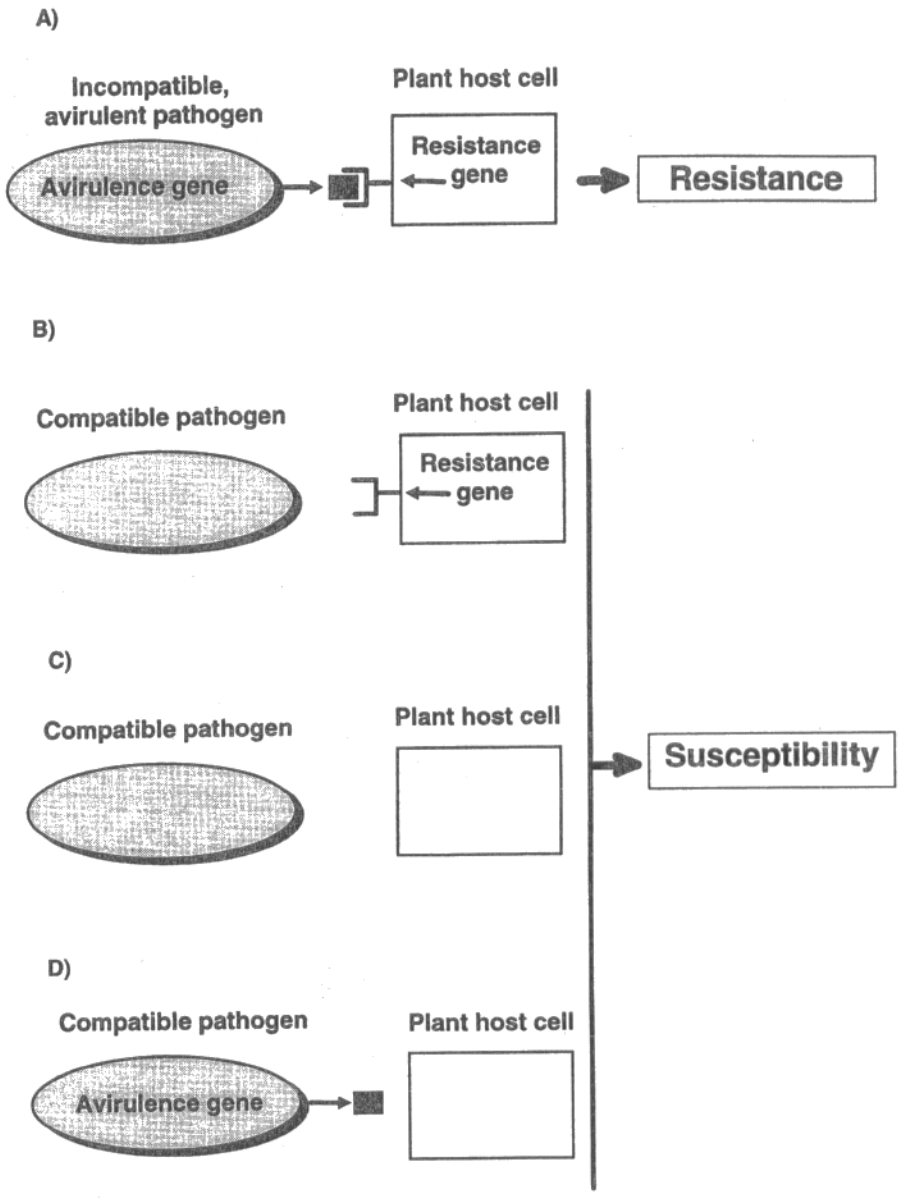

Figure 2. Molecular model of the gene-for-gene interaction (adapted from Staskawicz *et al.*, 1995). Resistance occurs only if there is a specific recognition between the resistance gene product and the product of the matching avirulence gene (A). In the absence of any recognition (B,C,D), no resistance reaction occurs and the pathogen can colonize the plant which results in a susceptible phenotype.

In an individual plant-pathogen system, there can be large number of plant resistance genes with different specificities for avirulence genes from the pathogen: more than 50 different specificities and therefore different resistance and avirulence genes have been

described for example for powdery mildew in barley or stem and leaf rust in wheat (Jørgensen, 1994; McIntosh *et al.*, 1995). Gene-for-gene interactions are typical for biotrophic pathogens (such as mildew and rust, Fig. 3) which depend on living cells of the host plant for their supply of nutrients. The race-specificity of their interactions with the host indicates a very specific biological interaction. In contrast, the necrotrophic pathogens which kill the plant cells and live from the nutrients released from the cells do usually not show race-specific interactions with the host plant. Examples of host-pathogen interactions for which a gene-for-gene relationship has been demonstrated is given in Table 1, which is by no means exhaustive.

Table 1. Incomplete list of pathosystems in crop plants for which the gene-for-gene relationship has been shown.

Plant	**Pathogen**
Triticum aestivum (wheat)	*Puccinia recondita* (leaf rust)
Triticum aestivum (wheat)	*Puccinia striiformis* (stripe rust)
Triticum aestivum (wheat)	*Puccinia graminis* (stem rust)
Triticum aestivum (wheat)	*Erysiphe graminis* f.sp. *tritici* (powdery mildew)
Hordeum vulgare (barley)	*Erysiphe graminis* f.sp. *hordei* (powdery mildew)
Zea mays (maize)	*Puccinia sorghi* (common rust)
Oryza sativa (rice)	*Xanthomonas oryzae* (bacterial blight)
Oryza sativa (rice)	*Pyricularia oryzae* (rice blast)
Malus sylvestris (apple)	*Venturia inaequalis* (apple scab)
Lycopersicon esculentum (tomato)	Tobacco mosaic virus (TMV)
Lycopersicon esculentum (tomato)	*Cladosporium fulvum* (leaf mold)
Solanum tuberosum (potato)	*Phytophtora infestans* (potato blight)
Solanum tuberosum (potato)	*Heterodera rostochiensis* (golden nematode)
Lactuca sativa/Serriola (lettuce)	*Bremia lactucae* (downy mildew)
Linum usitatissimum (flax)	*Melampsora lini* (flax rust)
Phaseolus vulgaris (French bean)	*Pseudomonas syringae* pv. *phaseolicola* (halo blight)

In addition to the cases described above where plant resistance is based on a specific interaction, there are also a number of cases where there is specific susceptibility to a pathogen due to a single gene in the host and the pathogen. Examples for such interactions can be found when a pathogen synthesizes a host-specific toxin. One example is the HV-toxin produced by *Helminthosporium victoriae* resulting in Victoria blight of oats (Ellingboe, 1976). There, the dominant *Vb* gene in oat is essential for sensitivity to the toxin and susceptibility to the disease. The ability of the pathogen to produce the toxin is under control of a single dominant gene in the pathogen. Only the combination of the presence of the *Vb* gene and the synthesis of the host-specific toxin results in a compatible, susceptible interaction. All the other possible gene combinations give an unspecific resistant, incompatible interaction. The selective pressure in this case is on mutational loss of an active *Vb* gene in the plant.

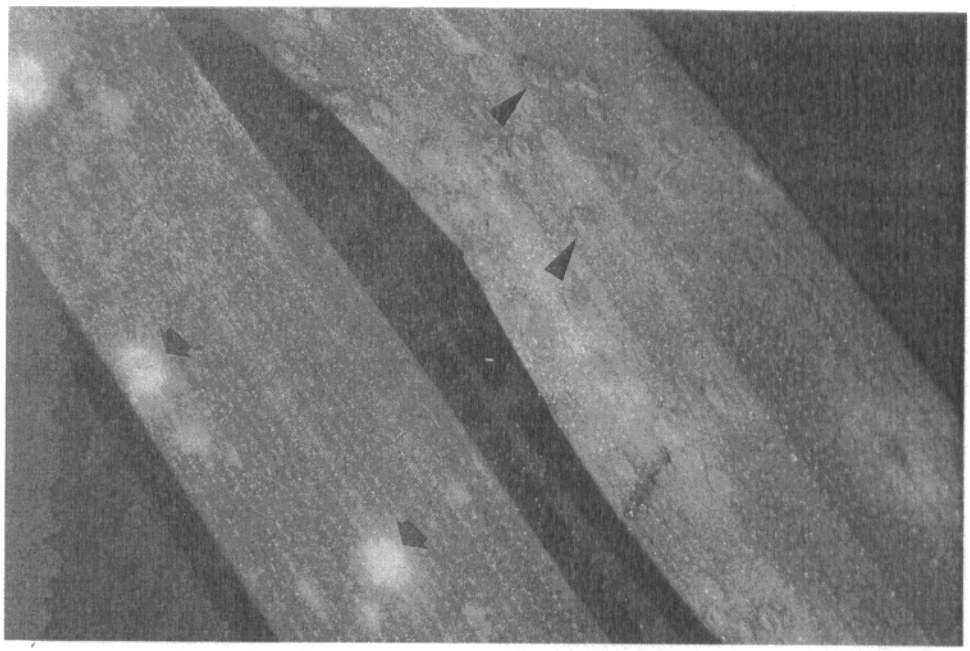

Figure 3. Phenotype of a typical resistance interaction conferred by a dominant resistance gene. The presence of the *Lr9* resistance gene of wheat against leaf rust results in a hypersensitive reaction after infection with an avirulent pathogen (arrows) wheareas a near-isogenic plant line without the resistance gene shows a compatible reaction and growth of the pathogen (arrowheads).

Gene-for-gene interactions were not only found between plants and biotrophic fungi but also in some instances with hemibiotrophic fungi (e.g. *Phytophtora, Colletotrichum*) nematodes, bacteria, insects and viruses. In tobacco, the resistance gene N' shows a race-specific reaction to different viral strains of the tobacco mosaic virus, i.e. some strains can spread systemically in the plant whereas the plant is resistant to other strains. This resistance trait is inherited in a Mendelian fashion as though it were conditioned by a single dominant gene. Similar race-specific resistance has been described against many bacterial pathogens such as *Pseudomonas syringae* pv. *glycinea* that causes leaf spot disease on the cultivated soybean and for which many different races are known (Fett and Sequeira, 1981). The resistance against the Hessian fly (*Mayetiola destructor*), an insect pest of wheat, was also shown to be based on a gene-for-gene relationship (Hatchett and Gallun, 1970). 25 resistance genes have been shown to be effective against the 13 reported biotypes of the Hessian fly (Patterson *et al.*, 1992). The common occurrence of gene-for-gene relationships suggests that there may be a common biological basis in the molecular recognition and signal transduction events involved in controlling resistance to diverse pathogens and pests. The recent finding that the products of resistance genes against bacterial, viral, fungal and nematode diseases have homologous domains (Bent, 1996) and can so far be grouped into only three different protein classes is a very nice confirmation of the ideas generated by classical genetics.

III. Genetic Analysis of Race-Specific Resistance Genes

A. DISEASE RESISTANCE GENES OCCUR IN ALLELIC SERIES AND AS GENE CLUSTERS

Classical genetic studies demonstrated that the same locus in different plant lines carried distinct alleles with different specificities to various pathogen races (Fig. 4A). In addition, resistance genes are often clustered at specific loci (Fig. 4B). In fact, it is genetically not easy to distinguish between true alleles at a resistance locus and a cluster of related genes at a particular chromosomal region. Crosses between plants with distinct resistance genes can help to clarify this question. If two genes are allelic, it will not be possible to get a chromosome with a combination of the two specificities (unless we assume the hypothetical event of an intramolecular recombination resulting in a combination of two different, specific resistances in the same gene). If the two specificities are due to two different genes, it is possible, at least theoretically if sufficient individuals in a segregating population are tested, to find recombinants. However, if the genes are physically very closely linked in a gene cluster of tandemly repeated genes, very large populations have to be built up and screened for the rare recombination events. This is often not feasible and therefore two distinct genes might be classified as alleles due to the very low recombination frequency between the two genes. Thus, alleles defined by classical genetic analysis might as well represent two closely linked genes in a molecular analysis.

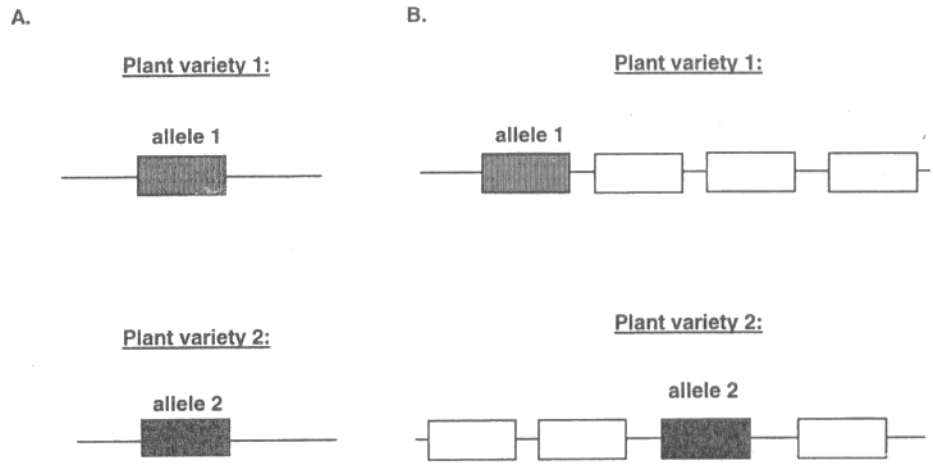

Figure 4. Schematic model for the organization of resistance gene loci as revealed by classical genetic analysis. (A) Alleles of the same gene from different plant lines are responsible for distinct race-specific resistances against a pathogen. (B) Physically closely linked genes are arranged as tandem repeats. Each of these genes may encode a gene product with a different specificity against a pathogen.

Several interesting cases of allelic series or clusters of resistance genes have been described. The *Rp1* locus in maize confers race-specific resistance to *Puccinia sorghi*,

the maize rust pathogen. The *Rp1* locus, located at the tip of chromosome 10, has at least 14 different alleles with distinct specificities called *Rp1-A* to *Rp1-N* (Saxena and Hooker, 1968). Several of these *Rp1* alleles have high natural mutation frequencies from resistance to susceptibility. Whereas normal mutation frequencies are about 10^{-6} to 10^{-7} per gene and generation, some *Rp1* alleles mutate at frequencies of 10^{-4} per generation. This high meiotic instability at the *Rp1* locus is probably due to unequal crossing-over (Sudupak *et al.*, 1992). It was also found that in all the tested cases the "alleles" at the *Rp1* locus were in fact distinct genes and recombinants with the combined specificities of the two parents could be found (Bennetzen *et al.*, 1994). Some of these progeny did not show flanking marker exchange as is expected for a normal, "symmetrical" recombination event, suggesting that genetic events such as gene conversion, intrachromosomal crossing-over or unequal sister chromatid exchange had occurred and contributed to the observed instability of the *Rp1* locus. The presence of a number of tandemly repeated copies of a very similar sequence, and the resulting unequal crossing-over events, might be the basis for the generation of new resistance specificities against the pathogen. Such a mechanism might help to compensate the potential advantage of the pathogen in its coevolution with the host plant: the pathogen with its large population size and usually fast generation time has to lose only a dominant function (i.e. the avirulence gene) to be able to grow on a previously resistant variety. In contrast, the plant must create a new dominant resistant gene to defend against such a new compatible race. Therefore, gene clusters of closely related genes might form the molecular basis for the rapid evolution of new specificities in the host plant.

In flax, 31 strain- or race-specific resistance genes have been characterized which confer resistance to different isolates of the flax rust pathogen *Melampsora lini*. They map to five distinct genetic loci, *K, L, M, N,* and *P* (Ellis *et al.*, 1988) of which the *L* and *M* locus have been particularly well studied. These two loci are also examples for two distinct strategies to evolve different specifities of resistance. Thirteen different resistance specificities map to the *L* locus. There have been many attempts to get recombinants between two different *L* locus specificities in coupling, but with no success. This suggests that, at the *L* locus, there is an allelic series of genes with different specificities. However, the genetic organization of the *M* locus is different. Seven different specific resistance genes map to this locus and recombination between different specificities was found. Obviously, the *M* genes are closely linked, tandemly repeated genes which span a genetic distance of around 0.5 centiMorgan. The relative position of four of the *M* genes was determined genetically. Flax is an ancient tetraploid species and molecular data indicate that the *L* and *M* locus are homologous, i.e. they correspond to the identical loci on the two original diploid genomes that were fused in flax (Ellis *et al.*, 1995). Obviously, the *L* and *M* loci have developed in two different ways: multiple alleles with different specificities evolved at the *L* locus whereas gene duplication, possibly followed by gene amplification through unequal crossing-over, occurred at the *M* locus. The initial duplication event might have occurred after transposon activity or by mispairing of two juxtaposed repeated sequences and subsequent non-homologous recombination. Thus, an initial duplication might have been the reason for the different evolution of the two resistance loci.

In lettuce, race-specific resistance genes are used for resistance breeding against the downy mildew pathogen *Bremia lactucae* and their genetics has been studied in detail. At least 13 different resistance genes (called *Dm* genes) have been described. These 13 genes map to four linkage groups. The group 1 gene cluster contains the genes *Dm* 1,2,3,6,14,15 and 16 which are tightly linked but show recombination and are therefore distinct genes (Farrara *et al.*, 1987). The simultaneous analysis of the genetics of avirulence in the pathogen make the lettuce-downy mildew system one of the best characterized gene-for-gene systems in plant pathology.

Another well studied resistance locus in plants is the *Mla* locus conferring resistance to powdery mildew in barley. In a recent review, 28 genes with different race-specificity were listed at the *Mla* locus (Jørgensen, 1994). These 28 genes are arranged as a large gene cluster. Recombination between some genes was detected (Wise and Ellingboe, 1985), demonstrating that not all the observed specificities result from different alleles of the same gene (thus we expect a situation as shown in Fig. 4B). It remains to be seen how many closely linked genes reside at this locus. It will be one of the most challenging research topics in the next years to analyse and study such complex loci at the molecular level. The large number of "alleles" at a single locus for different races of the same pathogen provides a unique opportunity to study the molecular basis of race-specific resistance.

B. GENETIC EVIDENCE FOR MOLECULAR SIMILARITY AMONG DIFFERENT RESISTANCE GENES

There is some evidence from classical genetic studies that resistance genes against several diseases might be similar and thus a small number of genes would form the basis of a superfamily of resistance genes that behave according to the gene-for-gene hypothesis. In wheat, the two resistance genes *Sr15* and *Lr20,* which confer resistance to stem rust and leaf rust respectively, map to the same locus and have never shown recombination. Additionally, mutagenesis experiments showed that simultaneous changes occurred in both specificities. This is strong evidence that the two genes are identical and that a single gene can confer resistance to the two different diseases (McIntosh *et al.*, 1995). In addition, *Lr20/Sr15* is completely linked with the *Pm1* powdery mildew resistance locus. Thus, resistance genes against the three fungal wheat diseases leaf rust, stem rust and powdery mildew are either identical, or alleles or belong to the same tightly linked gene cluster.

Evidence for the genetic relatedness of resistance also came from the observation that resistance genes from different plant species recognize the same avirulence determinant in a bacterial pathogen. In several cases the transfer of isolated avirulence genes between bacterial strains of *Pseudomonas syringae* or *Xanthomonas campestris* pathovars showed that the same avirulence gene was recognized by several plant species (Michelmore, 1995). It was also shown that *Arabidopsis*, bean and soybean all have a resistance gene that recognizes the *avrRpt2* avirulence gene (Kunkel *et al.*, 1993). It is likely that the molecular basis for the resistance against *avrRpt2* is based on very similar recognition processes. This classical genetic evidence for a similarity of resistance genes will be of importance for the isolation of resistance genes by homology with known resistance genes.