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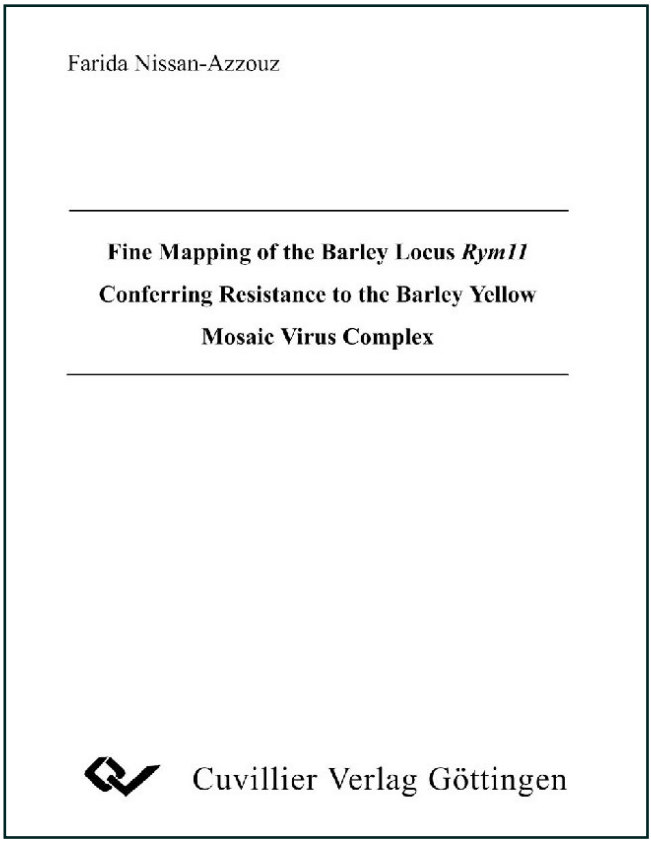
Fine Mapping of the Barley Locus Rym11 Conferring Resistance to the Barley Yellow Mosaic Virus Complex



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1 Introduction

1.1 Disease resistance in plants

1.1.1 Broad-spectrum resistance

Broad-spectrum resistance is a defence strategy that plants harbour against a large range of natural enemies such as bacteria, fungi, nematodes and viruses. Two types of broad-spectrum resistance, active and passive are distinguished. With respect to passive resistance, toxic compounds are constitutive independently of the presence of the pathogen (Osbourn et al., 1996). The toxic alkaloids in potato are an example of a passive resistance mechanism.

In case of active broad-spectrum resistance, defence reactions are initiated only when the plant is facing a pestilent attack; the effectiveness of such reactions is, nevertheless, against various potential pathogens. An example of active defence mechanism is the induced resistance (IR) that is activated e.g. upon an inducer-pathogen attack, enabling the plant to drive an effective defence response against a second attacking pathogen, the challenger. The first induced resistance is called local acquired resistance because it is limited to the infection site. The resistance may then spread systematically through the entire plant body leading to a broad-spectrum, systemic resistance (Ryals, 1996) such as the systemic acquired resistance (SAR). A set of genes known as SAR genes or pathogenesis related (PR) genes because of their implication in the production of the pathogenesis related proteins (PR-proteins) have been identified to be associated with SAR mechanisms in dicotyledonous species. However, it has not been clearly elucidated how the SAR is involved in monocotyledonous plants.

For plant improvement, broad-spectrum resistance has the advantage of being effective against several pathogen species. Breeding to increase the level of this resistance may be of great profit. However, the level of toxicity associated with the broad resistance mechanisms may have negative side effects particularly in food and fodder crops. For instance, it may be unacceptably high to humans and cattle, or decrease dramatically the nutritional value. Another disadvantage is that broad-spectrum resistance against a wide range of generalist pathogens and pests may be associated with increased attractiveness to specialist species (Niks and Lindhout, 2000).

1.1.2 Hypersensitivity resistance

The hypersensitive response (HR) is classically defined as a locally triggered cell death in the host plant at the site of attack by a pathogen (Agrios, 1997). Even hypersensitive resistance is, in many cases, associated with other active defence mechanisms, such as the PR-protein production, one particular aspect is that the HR effectiveness is race-specific. This enables the activation of the defence only to certain genotypes of the pathogen. For this reason, this type of resistance is also known as vertical resistance. In fact, a graph with many vertical columns is obtained when the reaction of resistance of a host plant genotype is plotted against a set of pathogen genotypes (VanderPlank, 1963).

To explain the race-specificity feature of hypersensitivity resistance, Flor (1971) proposed the hypothesis of a gene-for-gene interaction: 'For each gene conditioning resistance in the host, there is a specific gene conditioning pathogenicity in the parasite'. Flor made the emphasis on pathogenicity, literally virulence, but currently the emphasis is on avirulence. The resistance is determined by an interaction between the product of the resistance gene of the host plant and the product of the avirulence gene of the pathogen. A model elicitor/receptor has been suggested to elucidate the molecular basis of the gene-for-gene interaction (Keen, 1982).

In this model the dominant allele *R* for resistance produce a receptor molecule that recognizes an elicitor molecule produced by the dominant allele Avr of the avirulence gene. This recognition event elicits the hypersensitive reaction, which consists of a signal transduction pathway leading to a cascade of physiological reactions in the plant that are responsible for cell-death in the infection site (Blumwald et al., 1998). In case the pathogen carries the virulence allele, avr, rather than the avirulence allele, Avr, there will be no, or a mutilated, avirulence gene product and therefore, no recognition event will take place.

During the last years, the isolation of several *R* genes and the characterization of their products permitted considerable advances in the knowledge of the molecular basis of specific resistance. The comparison of different gene products revealed a strong sequence homology as well as five types of conserved structural domains: LRR (Leucine-Rich Repeats), Ser/Thr kinase (serine/threonine kinase), NBS (Nucleotide Binding Sites), LZ (Leucine Zipp) and TIR (Toll Interleukin Receptor). The *R* products seem to combine a receptor domain with an effector domain ensuring two main functions: the recognition of elicitor molecules thanks to protein-protein interaction

mechanisms and the direct or indirect activation of transduction signals (Hammond-Kosack and Jones, 1997). Furthermore, genetic and molecular studies demonstrated a particular genomic organization of *R* genes that display a tight genetic linkage to other genes or homologue sequences constituting, therefore, a complex locus also referred to as a cluster (Salmeron et al., 1996; Noël et al., 1999).

For plant breeding, hypersensitivity is the most widely used resistance thanks to the complete protection that it confers to the plant against the infection. Furthermore, the monogenic inheritance results in a high heritability of the resistance and leads to an easy selection. The crucial disadvantage is that hypersensitivity resistance is temporary effective because of the phenomenon of disease resistance breakdown.

1.1.3 Quantitative resistance

Quantitative resistance is a defence strategy that results in a reduced epidemic development despite a susceptible infection type. This resistance is race-non-specific, since the level of its effectiveness is equal to all the genotypes of the pathogen. It is besides of this feature, that the term 'horizontal' resistance was adopted. Indeed, when the degree of resistance of a host plant genotype is graphically plotted against a range of pathogen genotypes, a horizontal line is obtained (VanderPlank, 1963).

Quantitative resistance mechanisms are not well known because of the scarce number of studies performed to elucidate this phenomenon. However, they converge that processes such as plant cell wall penetration, growth and reproduction of the pathogen are less successful in comparison to a susceptible plant.

An interesting aspect of quantitative resistance is that it is often controlled by quantitative trait loci (QTLs) with small effects. This polygenic inheritance prevents the resistance from breakdown phenomenon and leads to a durability of the effectiveness. Therefore, quantitative resistance may represent an attractive alternative to hypersensitive response in plant improvement, but in the same time makes breeding more complicated because of the low heritability of quantitative traits. Moreover, the level of quantitative resistance is insufficient to protect crop products as ornamentals and leafy vegetables that should remain completely blemish-free to satisfy the quality demand of the market (Niks and Lindhout, 2001). In the future, and judging by the number of QTL mapping studies undertaken these last years (Young, 1999), it is no doubt that this type of resistance will occupy a major place of choice in breeding strategies.

1.2 Fine mapping: a prerequisite for resistance gene cloning

Three strategies have been adopted for resistance gene cloning in plants. For the genes with known products, the useful way for their isolation is the utilization of the homology-based cloning method. It consists of the development of degenerate oligonucleotides encoding a conserved amino acid sequence, and to use them in genomic library screening for identifying the responsible gene (Kanasin et al., 1996). The isolation of resistance genes with unknown function is carried out in two ways: by transposon tagging and positional cloning. With respect to transposon tagging, an insertional mutagenesis is achieved exploiting the capacity of transposable elements to inactivate a target gene by insertion into it. Moreover, transposable elements can be introduced in other species using transformation techniques. For example, the isolation of the tomato gene *Cf-9* conferring resistance to *Cladosporium fulvum* has been carried out by transposon tagging using the maize transposon system *Ac/Ds* (*Activator-Dissociation*) (Jones et al., 1994). For barley, evidence that the Ac/*Ds* system could be used to tag genes of interest was given by Koprek et al. (2000).

The other alternative to isolate uncharacterised resistance genes is positional cloning also known as map-based cloning. It consists in isolating the gene of interest by linked markers. Two variants of this strategy were developed. The classical 'chromosome walking' approach (Rommens et al., 1989) consists in the identification of DNA markers flanking the target gene on both sides. Therefore, the clone containing the nearest marker is used in genomic library screening to start the isolation of an overlapping clone series until the flanking marker on the opposite side is detected. Because higher plants possess, in general, a large genome and a high frequency of repetitive DNA, this approach is time consuming and, thus, presents serious limits. As an alternative, Tanksley et al. (1995) suggested the 'chromosome landing' strategy. The idea is to search for so tightly linked flanking markers that the physical distance of the target interval would be less than the average size of the YAC or BAC clones to be probed. For example in the case of YACs, the flanking markers should be localised less then 200 kb from the target. Therefore, it would be possible to land directly on the clone containing the gene without searching for overlapping clones. The identification of tightly linked markers, which corresponds to the fine mapping of the target gene, is a fundamental stage in gene cloning. The steps of this procedure can be resumed as below.

Fine mapping

The high density molecular maps available today for most cultivated species might be useful tools for starting the identification of linked markers. Therefore, it is possible to proceed to a marker saturation of the targeted region combining high-throughput marker techniques with regional targeting strategies that permit the analysis of a particular genomic fragment. Two types of genetic materials can be used, i.e. near isogenic lines (NILs) and pooled DNA also referred to as bulked segregant analysis (BSA).

The NIL approach (Young et al., 1988; Martin et al., 1991) consists in using lines genetically identical except for the fragment containing the target gene. These lines result from backcross selection, as a gene is introgressed from a donor to a homozygous line. Upon a backcross series, the improved line is genetically similar to the recurrent parent except the introgressed gene region. Thereby, the recurrent parent together with the selected line constitutes a pair of NILs.

In the case of the BSA strategy (Michelmore et al., 1991), a population such as an F_2 generation that segregates for all parental alleles is considered. When the DNA of a set of individuals selected from this population is pooled in two bulks contrasting for the character of interest, the bulks are expected to contain the same alleles for the entire genome except for those linked to the gene encoding the character, which will consequently contrast. For the NILs as for the BSA bulks, the genetic differences can be analysed by high throughput markers such as AFLPs and RAPDs.

After identifying a set of linked markers, it is crucial to determine their order on the genetic map, so that those most closely linked to the gene can be selected for genomic library screening. The difficulty is to localize markers within physical distances corresponding to the library inserts size; for this it is necessary to map at 0.1 cM resolution, or even less. Indeed, genetic mapping is based on the crossing over frequencies observed between pairs of loci. These frequencies are lowest according to the degree of linkage. At 0.1 cM resolution, the detection of one crossing over event with a probability of 0.95 would drag the analysis of 3,000 individuals (Tanksley et al., 1995; De Vienne et al., 1998). The screening of a population containing thousands of individuals is time consuming. To facilitate this demanding task the flanking marker technique was suggested. It consists in mapping two markers flanking the gene at both sides, on a large population. The other markers are, then, mapped only on a subset of individuals carrying recombinations in the target interval outlined by the flanking markers. In this way, those closest to the gene are more efficiently identified.