Function of Genetic Material Responsible for Disease Resistance in Plants

By Gerhard Wenzel

1. Introduction

Today, the availability of recombinant DNA techniques together with advances in molecular biology and cell culture provides access to a **refined understanding of the genome.** Our present century is moulded by the invention of the genome structure and the subsequent use of this knowledge in genetics and its applied wing: breeding. Starting with the rediscovery of the Mendelian laws, classical segregation analysis and cytology formed the basis of scientific breeding strategies. Complete DNA sequences of many prokaryotes have been determined, the genome of yeast has been sequenced and it is expected that the base pairs of the model plant *Arabidopsis* will be sequenced before the end of this century. Although such a **sequence analysis provides the most complete information** about the genetic basis, it does not inform about the **meaning and the functionality of genes.** Taking into consideration the enormous size of the genome and the fact that a tremendous number of the base pairs are silent, it seems recommendable to analyse only those parts containing information.

To find such areas several approaches have been elaborated, making use of phenotypic segregations and correlations of such phenotypes to molecular linkage maps. A compromise is the analysis of DNA fragments generated by restriction enzymes gearing the development of this research area. Progress in analysing the higher plants' genomes is driven by two goals: (1) finding DNA probes closer and closer linked to a phenotype, and (2) making these selection tools so easy that they can be used under applied aspects.

The increasing amount of information documented in **dense gene maps** together with an excellent **bioinformation system** allows increasingly calculations about the function of genes (Michelmore 1995; Jones 1996). Particularly under the aspect of **synteny,** comparisons will be possible, probably elucidating common principles, e.g. in defence mechanisms against pests, or in the development of morphological structures. In the area of secondary product formation already a wide range of information about the biochemical pathways exists (Henry et al. 1996) and increasingly the corresponding genes are grouped to the responsible en-

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zymes. Two areas are still unclear: (1) the molecular architecture influencing plant yield, the morphology or the function of sexual organs, and (2) the function of genes responsible for resistances. Since the first topic is covered by Thießen and Saedler (this Vol.), this chapter will focus on the advances **in mapping and understanding the functionality of genes responsible for disease resistance.**

Breeders have used monogenic disease resistance R-genes in their efforts to produce resistant varieties. The R-genes enable plants to recognize specific races of pathogens and to react with a specific defence response. However, races of pathogens with new virulences evolve that can overcome individual R-genes. Since now the structure of increasing numbers of such R-genes is elucidated, progress related to a functional understanding of the host pathogen interaction is anticipated. Here, the development will be discussed under the aspects of how and where a contribution of the research to the function of genes responsible for resistance is growing.

2. The Technique

Diversity at the phenotypic level is caused by corresponding differences in the DNA sequence. The availability of recombinant DNA techniques provides access to a refined analysis of the genome. Point mutations, insertions, deletions or inversions cause differences in the nucleotid sequence and variability in the length of individual restriction fragments. The altered fragment lengths can be detected by gel electrophoresis – or most recently also by optical means (Anantharaman et al. 1977) – and result in restriction fragment length polymorphisms (RFLPs). **The procedures used for the genome and gene identification include chromosome walking, megabase techniques, as well as tagging and c-DNA approaches.**

RFLP probes may be converted into sequence tagged sites (STS; Blake et al. 1996) or specifically cleaved amplified regions (SARS; Paran and Michelmore 1993). This allows direct visualization of the DNA in the gels without the need of labeling; an important advantage in applied work.

The RFLP method advanced to the very powerful amplified fragment length polymorpism (AFLP) technique (Vos et al. 1995), allowing much denser maps by identifying very small differences in the genome. Another approach, the randomly amplified polymorphic DNA sequences (RAPD) (Williams et al. 1991), is based on genomic DNA fragments bordered by defined primers which are amplified during a polymerase chain reaction (PCR).

The RAPD technique can be performed with any conceivable primer sequence comprising about ten nucleotides and containing approximately 50% cytosine and guanine residues. While the advantage of this technique is that polymorphisms can be detected directly upon size fractionation on a gel without expensive and time-consuming hybridi-

zation procedures, the reliability in different genetic backgrounds is only limited. For RAPDs the problem appears that dominant markers show repulsion linkage to the resistance gene. In those cases the RAPD marker has to be transferred into sequence characterized amplified regions (SCARs), e.g. in pea for the *Erisiphe pisi* resistance (Dirlewanger et al. 1994). An exception is the RAPD marker for *Vertillicum* in tomato: it is codominantly inherited and directly differentiates between the resistant and susceptible allele (Kawchuck et al. 1994).

Additionally, **microsatellites**, small conserved base sequence patterns distributed rather evenly over the genome, can be incorporated in those instances where no other polymorphisms are detected (Hearne et al. 1992). While RFLP, RAPD and AFLP marker systems depend predominantly on anonymous DNA sequences, the microsatellite technique uses defined sequence motives of two or four base pairs. In eukaryotic genomes microsatellites express a highly dispersed distribution. By PCR the different sizes of microsatellite loci can be easily detected. Due to their high amount of information, they are a useful marker system, especially for species with low genetic diversity, e.g. wheat (Röderer et al. 1995). These new marker techniques, particularly the microsatellites and AFLPs, have not yet been used for marker-aided selection, but for producing dense maps aiming at the identification of genes by chromosome landing (Tanksley et al. 1995).

Since in gene identification the application of molecular markers demands the need to know the localization of the marker in the genome, **genetic linkage maps** are an additional prerequisite for the localization and the analysis of gene functions. Often for a securer gene identification, the bulked segregant analysis (BSA), doubled haploids (DHs), nearly isogenic lines (NILs) or recombinant inbreed lines (RILs) are used as mapping populations.

3. Presently Mapped Major Resistance Genes

For the world's most important plant pathogens, the fungi, up till now progress in applying molecular procedures has been rather slow. The understanding how the **host/pathogen** interaction works is a prerequisite in order to start unconventional breeding programmes. The presently possible **transformation** of genes for the expression of antifungal proteins or for an overexpression of phytoalexins is normally not sufficient to protect the plant under field conditions (Hain et al. 1993). Thus, additional knowledge is needed.

The techniques to identify and use viral genes, e.g. the viral coat protein gene, the antisense RNA for virus resistance, the movement proteins and the replicase mediated resistance, as well as the strategies against bacterial pathogens are already summarized by Horn et al. (1996) in a previous volume.

Table 1. Summary of dicot host plants and diseases for which DNA probes are available, together with a reference where further information can be found

Host	Disease	Number of alleles identified	Technique	Reference
Arabidopsis	*Pseudomonas syringae*	2	Tagging, RFLP	Simonich and Innes (1995)
	Peronospora parasitica	5	RAPD, STS	Tör et al. (1994)
Beans	*Colletrochum lindemuthianum*	1	NIL, RFLP, SCAR, RAPD	Adam-Blondon et al. (1994)
	Common bean mosaic virus	1	NIL, RAPD	Haley et al. (1994)
	Uromyces appendiculatus	3	NIL, RAPD	Johnson et al. (1995)
Cucumber	*Cladosporium cucumerinum*	1	RFLP	Kennard et al. (1994)
	Pseudoperonospora cubensis	1	RFLP	Kennard et al. (1994)
Flax	*Cochliobolus carbonum*	3	Tagging	Lawrence et al. (1995)
Lettuce	*Bremia lactuca*	19	BSA, STS, RAPD, RFLP	Kesseli et al. (1994)
	Plasmopara lactucae	1	RAPD	Robbins et al. (1994)
	Turnip mosaic virus	1	BSA, RAPD	Robbins et al. (1994)
Pea	*Erysiphe pisi*	2	RIL, RFLP, RAPD, SCAR	Dirlewanger et al. (1994)
	Fusarium oxysproum	1	RFLP, Microsatellite	Dirlewanger et al. (1994)
	Pea mosaic virus	1	RFLP, Microsatellite	Dirlewanger et al. (1994)
	Seed-borne mosaic virus	1	RFLP, RAPD	Timmerman et al. (1993)
Potato	*Globodera rostochiensis*	3	NIL, RFLP, RAPD, AFLP	Ballvora et al. (1995)
	Phytophthora infestans	2	DH, BSA, RFLP, AFLP,	El-Karbotly et al. (1996)
			QTL, RFLP	Leonards-Schippers et al. (1994)
	Potato virus X	2	RFLP	Ritter et al. (1991)
	Potato virus Y	1	RFLP	Hämäläinen et al. (1997)

Table 1 (continued)

Host	Disease	Number of alleles identified	Technique	Reference
Rape seed	*Plasmodiophora brassica*	1	RFLP, AFLP	Voorrips et al. (1997)
Soybean	*Heterodera glycines*	1	RIL, RFLP	Webb et al. (1995)
	Phytophthora megasperma	3	NIL, RFLP	Diers et al. (1992)
	Soybean mosaic virus	1	Microsatellite, RFLP	Y.G. Yu et al. (1996)
Sugar beet	Rizomania	1	RFLP	Barzen et al. (1995)
	Heterodera schachtii	3	RFLP, RAPD	Salentijn et al. (1995)
	Beet necrotic yellow vein virus	1	RAPD	Scholten et al. (1997)
Tomato	*Cladiosporium fulvum*	6	NIL, BSA, RFLP, AFLP	Thomas et al. (1995)
	Fusarium oxysporum	3	NIL, RFLP	Sarfatti et al. (1991)
	Leveillula taurica	1	RFLP	Chungwongse et al. (1994)
	Macrosiphum euphorbiae	1	RFLP, RAPD, STS	Kaloshian et al. (1995)
	Meliodogyne incognita	2	NIL, RFLP, RAPD	Yaghoobi et al. (1995)
	Oidium lycopersicon	1	BSA, RFLP, RAPD	van der Beek et al. (1994)
	Verticillium dahliae	1	NIL, RAPD	Kawchuk et al. (1994)
	Potato virus X	1	RFLP	Ritter et al. (1991)
	Tomato spotted wild virus	1	NIL, RFLP, RAPD	Stevens et al. (1995)
	Tomato mosaic virus	1	RFLP	Tanskley et al. (1992)
	Tomato yellow leaf curl virus	1	RFLP	Zamir et al. (1994)

Table 2. Summary of monocot host plants and diseases for which DNA probes are available, together with a reference where further information can be found

Host	Disease	Number of alleles identified	Technique	Reference
Barley	*Erysiphe graminis*	10	NIL, RFLP, DH,	Schönfeld et al. (1996), Büschges
		QTL	AFLP	et al. (1997), Backes et al. (1997)
	Puccinia gramminis	2	DH, BSA, RFLP	Kilian et al. (1995)
	Puccinia hordei	1	BSA, RAPD	Poulsen et al. (1995)
	Puccinia striiformis	QTL	RFLP	Chen et al. (1994)
	Rhynchosporium secalis	4	DH, NIL, RFLP	Graner and Tekauz (1996)
		QTL	DH, RFLP	Backes et al. (1995)
	Pyrenophora teres	1	DH, RFLP	Graner et al. (1997b)
		QTL	DH, RFLP	Steffenson et al. (1996)
	Cochliobolus sativus	1, QTL	RFLP	Steffenson et al. (1996)
	Typhula incarnata	1	RFLP	Graner et al. (1997a)
	Pyrenophora graminea	QTL	RFLP	Pecchioni et al. (1996)
	Barley yellow dwarf virus	1	RFLP	Collins et al. (1996)
	Barley stripe mosaic virus	1	DH, RFLP	Edwards and Steffenson (1996)
	Barley yellow mosaic virus	7	DH, RFLP, RAPD	Graner (1996)
	Heterodera avenae	1	RFLP	Langridge (cit. In Graner 1996)
Maize	Maize dwarf mosaic virus	2	RFLP	Ming et al. (1997)
	Bipolaris maydis	1	RFLP	Zaitlin et al. (1993)
	Puccinia sorghi	1	NIL, RFLP	Hulbert and Benetzen (1991)
	Cercospora zeae-maydis	QTL	RFLP	Bubeck et al. (1993)
	Collerotrichum graminicola	QTL	RFLP	Jung et al. (1994)
	Helminthosporium trucicum	1	NIL, RFLP	Bentolia et al. (1991)

Table 2 (continued)

Host	Disease	Number of alleles identified	Technique	Reference
Oats	*Puccinia coronata*	1	BSA, RAPD	Penner et al. (1993a)
	Puccinia graminis	1	NIL, RAPD	Penner et al. (1993b)
Rice	*Orseolia oryzae*	1	RIL, BSA, RFLP, RAPD	Mohan et al. (1994)
	Pyricularia oryzae	3	NIL, RFLP	Miyamoto et al. (1996)
	Pyricularia grisea	2	NIL, RFLP	Z.H. Yu et al. (1996)
	Rice tungro virus	1	RFLP, RAPD	Sebastian et al. (1996)
	Xanthomonas oryzae	6	NIL	Wiliams et al. (1996)
Sorghum	*Sporoisorium reilianum*	1	RFLP, RAPD	Oh et al. (1994)
Wheat	*Heterodera avenae*	2	BSA, NIL, RFLP, RAPD	Eastwood et al. (1994)
	Mayetiola destructor	11	RFLP	Dweikat et al. (1997)
	Puccinia recondita	3	RFLP, RAPD, STS	Feuillet et al. (1995)
	Puccinia graminis	1	RFLP	Paull et al. (1994)
	Erysiphe graminis	8	NIL, RFLP	Hartl et al. (1995)
	Wheat streak mosaic virus	1	STS, RAPD	Talbert et al. (1996)

As a first central step in the direction of identifying resistance genes, during recent years a rapidly increasing number of monogenic, race-specific genes showing **gene-for-gene interaction** have been mapped in economically important dicot (Table 1) and monocot (Table 2) species. This demands the production of mapping populations and the skills for exact phenotypic evaluations.

For host solidus pathogen interactions with fungi imperfecti, e.g. for beans and *Colletotrichum lindemuthianum*, the proof of a gene-for-gene interaction is missing of course. Due to the formation of races and the independent inheritance of seven dominant genes for resistance against this fungus in beans, Adam-Blondon et al. (1994) grouped a gene (*Are*) to the category of race-specific genes. They even found closely linked markers in NILs, which now can be used for marker-aided selection. Sometimes the genes identified are not resistance genes against the pathogen itself, but for a vector, e.g. the gene for rice tungro virus resistance which does not act against the virus but rather causes insect resistance against *Tetigonia viridissima*, the responsible virus vector (Sebastian et al. 1996).

Besides the race-specific genes, an increasing number of **quantitatively inherited genes**, quantitative trait loci, (QTLs) are localized (Dirlewanger et al. 1994; Backes et al. 1995, 1997). The identification of polygenes for disease resistance is not different from the identification of other polygenic traits. For review of the basic principles see Tanksley et al. (1995). Some characterizations of polygenic traits are incorporated in Table 1 and 2. To find correlations between geno- and phenotype the progeny is divided into several subpopulations depending on the allele groups of a trait (e.g. parental type, heterozygotes). A linkage between a QTL and a genetic marker is given when the phenotypic means of a class of markers are significantly different. The most commonly used procedure for mapping QTLs is today interval mapping according to Lander and Botstein (1989), where chromosome segments flanked by two markers are analysed. For the identification of gene functions, presently only the race-specific genes are under investigation.

4. Genomic Organization of Resistance Genes

Although the knowledge about the number and genetic localization of disease resistance genes is still incomplete, the knowledge on the genomic organization of the first genes is rapidly growing. Evidently the **resistance genes are not evenly distributed along the chromosomes** but rather tend to **form clusters**. These are either composed of different specificity or of genes that condition resistance against one pathogen. The presence of heterospecific clusters has been described for tomato, wheat (Ellis et al. 1995) and barley (Graner et al. 1996). Homospecific clusters are more common (Mahadevappa et al. 1994). In barley particularly the *Mla* locus represents an extreme example of multiple allelism

(Jahoor et al. 1993). Examples of other complex resistance genes have been studied extensively in flax where the *L* locus confers resistance to rust exhibiting multiple allelism.

The physical analysis of the gene sequence reveales that its 3' region consists of a stretch of tandem repeated motives, the repeat number of which differs in the alleles analysed (Ellis et al. 1995). It seems that variability in repeat number results in the generation of a new allele with altered specificity. Thus, one may speculate that the genetic variability of the *Mla* locus in barley is accounted for by a similar mechanism. Differences may be also a result of gene amplification of an ancestral gene by unequal crossover events mediated by flanking repetitive elements (Ellis et al. 1995). Such questions may be solved by isolating the gene and subsequently identifying its functions.

5. Gene Isolation

After gene mapping, for gene identification, marker-based chromosome walking techniques are applied predominantly. Additionally, **tagging techniques using increasingly transposon induced mutant populations** (Osborne and Baker 1995) together with cDNA approaches are gaining importance under this aspect. For the walking technique high resolution maps have been constructed, allowing the saturation of the relevant chromosomal region with very closely linked markers. The closest ones will be used to select homologous clones from large insert libraries which in turn allow the construction of physical maps around the genes. An alternative strategy for the isolation of disease resistance genes exploits the observation that many resistance genes isolated in one plant species **share similar sequences or represent members of comprehensive and widespread gene families.**

Thus, isolation and mapping of homologous clones may lead to identification of candidates, which have to be further tested by genetic analysis. Particular examples for this approach are heterologous probes from plants like *Arabidopsis* or rice with small genomes but a huge amount of information available.

For resistance genes, common features like genes for **enzymes rich in leucine** (leucine-rich repeats, **LRRs**) or enzymes responsible for signal transductions are of a very great help. The information available – though still very limited – allows first speculations on the type of function of the genes identified.

6. Genes Presently Cloned

Eighteen genes responsible for disease resistance have been cloned up till now (Table 3). All are following the gene-for-gene hypothesis, and

Table 3. Common structural characteristics of proteins of cloned genes for resistance

Group	Protein	Host/pathogen	Structure	Reference
I	PTO	Tomato/*Pseudomonas*	Intracellular serine/threonine kinase membrane bound	Martin et al. (1993)
	PTI1	Tomato/*Pseudomonas*	Serine/threonine kinase phosphorylated by PTO, interacting with PTO	Zhou et al. (1995)
IIa	RPS2	*Arabidopsis/Pseudomonas syringae*	Intracellular protein with leucine zipper, nucleotide binding site, leucine-rich-repeats	Bent et al. (1996) Mindrinos et al. (1994)
	RPM1 PRF	*Arabidopsis/Pseudomonas syringae*		Grant et al. (1995) Salmeron et al. (1996)
IIb	N	Tobacco/TMV	Intracellular protein 1L-1R homology, nucleotide binding site	Whitman et al. (1994)
	L2, L6, L10 RPP5 RPP14	Flax/*Cochliobolus carbonum* *Arabidopsis/Peronospora parasitica*	leucine-rich repeats	Lawrence et al. (1995) Parker et al. (1996) Jones et al. (1996)
III	Cf-2 Cf-4 Cf-5 Cf-9 I2	Tomato/ *Cladosporium fulvum* Tomato/ *Fusarium oxysporum*	Transmembrane proteins with extracellular leucine rich repeats	Jones et al. (1996) Jones et al. (1996) Jones et al. (1996) Jones et al. (1996) Jones et al. (1996)
IV	Xa21	Rice/*Xanthomonas oryzae*	Transmembrane protein with intracellular kinase and extracellular leucine-rich repeat	Song et al. (1995)
V	Mlo	Barley/*Erysiphe graminis*	Transmembrane proteins nuclear localized	Büschges et al. (1997)

can be grouped into four sections according to the possible function of the proteins resulting from their DNA sequence. Most of these proteins are incorporated within the **ligation and/or in the signal transduction** (De Wit 1995). Fifteen encode an LRR motif. **This motif could not only explain recognition specificities but also allow their rapid evolution.** R-gene products might be explained to have two functions: molecular recognition and activation of plant defence upon recognition. The products may fall again into two classes, recognizing either **extra- or intracellular pathogen derived ligands.** It is, however, not yet clear whether the R-gene products interact directly with the avirulence gene (*Avr*-gene)-coded elicitors, or whether the subcellular localization can be deduced for their primary sequence. Furthermore, type and number of additional plant genes which are necessary for the signal transduction of the R-gene are unknown. **It is striking that R-genes for a wide range of pathogens of different plant species code for structurally similar proteins.** This similarity makes probable a high amount of mechanical conservation of the signal transduction chains used for the induction of reaction against pathogens (Bent 1996).

a) Intracellular Protein Kinase (Group I)

A common feature of proteins of group I is the **membrane bound serine solidus threonine kinase.** The first described and dominating example is the gene *Pto* of tomato causing resistance against the bacterium *Pseudomonas.* It codes a functional serine solidus threonine kinase (Loh and Martin 1995a,b). Subsequently, a second gene of the *Pto*-gene family, linked in a 400-kb region, was isolated causing sensitivity against the insectizide fenthion. It is also a serine/threonine kinase (Rommens et al. 1995). This fenthion sensitivity (*Fen*) gene expresses at the protein level 80% identity to *Pto.* Neither possess a region pointing to an extracellular or transmembrane localization but a possible site for membrane association in a number of proteins including protein kinase (Grand 1989). Consequently, both proteins can act with a postulated membrane bound receptor (Loh and Martin 1995a), and express homology to different serin/threonin kinases, including the transmembrane protein S-receptor kinase (SRK6) from *Brassica.* This probably codes for a receptor kinase coupled together with the *S*-locus-glycoprotein (SLG), a glycoprotein of the cell wall coded by the incompatibility locus *S.*

The S-incompatibility is located in the papillar cells of the stigma which detects probably specific structures of the pollen surface causing self-incompatibility in *Brassica.* This incompatibility is based on a gene-for-gene reaction like the *Pto/avrPto* interaction (Dickinson 1996).

Zhou et al. (1995) isolated using the yeast two hybrid system (Fields and Song 1989) the additional serine/theonine kinase, PTO-interacting (PTI1) which is phosphorylated by PTO. The authors proposed a hypothetical signal transduction chain, in which at first **an elicitor produced by an avirulent bacterium interacts directly or indirectly with PTO and then phosphorylates via PTI transcription factors**, which activate disease-relevant genes. By the resulting production of a protein the hypersensitive reaction is started.

Since *Pti* belongs to a gene family a protein homologous to PTI might exist, which is activated by FEN using the same transduction pathway. In the meantime, further PTO-interacting proteins were isolated showing homologies to transcription factors of tobacco (Bent 1996).

These proteins are similar to DNA binding proteins, identifying a conserved sequence at the promotors of pathogen-related (PR) protein genes. A PR box binding has been varified experimentally, which hints at a mechanism for expressing disease-relevant proteins coupling by this the detection of an avirulent pathogen with the expression of resistance genes.

b) Intracellular Proteins with a Nucleotide Binding Site (NBS) and C-Terminal LRRs (Group II)

The next three groups have the character LRR in common. LRRs might provide a general mechanism for providing both a regular protein structure on which to elaborate recognitional specificity and a DNA structure that because of these LRRs might have the capacity to rapidly evolve new specificities by unequal crossing over (Jones et al. 1996). Furthermore, LRRs are common proteins that interact with other proteins. **It is not unreasonable to consider their role as analogous to that of the antibody variable domain.** However, in plant selection for useful recognitional specificities this is only exercised germinally, unlike somatic selection exercised on the diversity generated in the mammalian system.

An LRR defines a turnable protein binding domain with 24 to 26 amino acids (Kobe and Deisenhofer 1994). They often appear in repeats of 1 to 40 and in functional and evolutionary different protein families, all of which are part of a protein-protein interaction and normally also part of signal transduction pathways, e.g. the transmembrane kinase (TMK1) or the receptor-like kinase (RLK5) in *Aradibopsis* (Walker 1993) or an LRR protein of unknown function detected in tomato with increased concentrations in infected plants (Toreno et al. 1996).

It is assumed that the LRR domain is the recognition and binding site perhaps of Avr proteins. **The specificity oft the LRRs depends** probably

less on the conserved repeatedly appearing hydrophobic groups present in the inner protein and responsible for tertiary structure but rather **from the interspersed exposed variable amino acids** (Bent 1996). The organization of repeated sequences and the appearance of defect mutants as a consequence of intragene rearrangements or point mutations in R-genes in these domains (Grant et al. 1995; Parker et al. 1996) are hints at **how on the molecular level new configurations and in consequence new specificity may appear.**

The presence of a nucleotide binding site makes it probable that the **resistance genes of group II need ATP or GTP for their function** (Traut 1994). This hypothesis is backed by preliminary results of directed mutagenesis of the NBS-consensus sequence which eliminates the hypersensitive reaction (HR) induced by RPS2 and N (Bent 1996). The function of the NBS domain is still unknown. It is possible, however, that an alteration of the interaction of R-proteins with other members of the signal transduction chain is responsible.

The resistance genes *Rps2, Rpm1* and *Rpp5* from *Arabidopsis*, the N-gene from tobacco mosaic virus (TMV)-resistant tobacco, *L6* from flax and *Prf* from tomato against different bacterial, viral and fungal pathogens; despite these differences, they have in common to code for cytoplasmic proteins which contain LRRs, and an NBS often called a P-loop. Within this group of R-genes the genes *Rps2, Rpm1* and *Prf* form a subgroup since they have in common a heptameric repetitive sequence motive, the so-called leucine zipper between N-terminus and the NBS and LRR domain.

This consensus sequence improves the protein-protein interaction and allows the homo- and heterodimerization of eukaryotic proteins (Alber 1992). It is not understood which role it has for the function of the R-genes, but experiments using the yeast-two-hybrid system (Fields and Song 1989) for the search of interacting compounds for the R-proteins are in progress (Bent 1996). The *Rpm1*-gene is active against two independent avirulence genes, ***avrRpm1*** and ***avrB*** of ***Pseudomonas syringae*** pv. ***maculicola*** or pv. ***glycinea***. If ***Rpm1*** codes for a receptor, it should be probable that only one or overlapping binding sites are responsible for both avr-gene products, since no mutants of *Arabidopsis* were found separating both specificities. Alternatively, a double specificity by the interaction of RPM1 with a general avr-receptor might be the reason (Grant et al. 1995). For the gene products of the two avirulence genes *avrRpm1* and *avrRpt2* from *Pseudomonas syringae*, a common factor is proposed for which both compete. This would also explain the epistasy of the two *avr*-genes (Reuber and Ausubel 1996; Ritter and Dangl 1996).

Since RPS2 is located in the cytoplasm demonstrated by mutation of a responsible membrane domain and RPM1 is not transmembrane, **the responsible factor might be a membrane protein acting between the extracellular *avr*-gene products and the intracellular R-gene products** (Innes 1996). A possible candidate is the bacterial protein harpin, coded by the *hrpZ* gene secreted from the bacterium. When it is injected to tobacco, necrosis is induced (He et al. 1993; Huang et al. 1995). However,

also the interaction of the bacterial avr-B-protein with the corresponding product of the resistant plant within the plant cell explains why at least some products of the resistance gene are located in the cytoplasm (Gopalan et al. 1996).

To group IIb belong the proteins N, L2, L6, L10, RPP5 and RPP14.

They have homologies to the cytoplasmic domains of the interleukin-1-receptor (IL-1) of mammals and the Toll-transmembrane protein from *Drosophila*, both inducing a signal transduction path resulting in the activation of immune genes in mammals and the function of the dorso-ventral polarity in the embryo of *Drosophila* (Dangl 1995). Recent studies demonstrated that a Toll/IL-1R-signal transduction path improves the immune response of adult flies after pathogen attack. It results in the transcriptional activation of genes for antimicrobial peptides (Lemaite et al. 1996).

It is possible that the conserved N-terminal part of the resistance genes *N*, *L6* and *Rpp5* is an effector domain, inducing a Toll-similar signal cascade (Parker et al. 1996). From *N*, *L6* and *Rpp5* reduced transcripts can be produced by alternative splicing or by the expression of a variant gene which consists only of the Toll homologous N-terminal part and the NBS. As proposed for truncated N, such transcripts might function as dominant regulators of resistance proteins via the stochastic binding of nucleotides (Dinesh-Kumar et al. 1995). The anticipated intracellular localization of N is understandable, since the life cycle of TMV happens in the cytoplasm of the host cell. In contrast to N, the proteins L2, L6 and L10 might be signal peptides secreted by the responsible gene product (De Wit 1995; Lawrence et al. 1995).

The *Rpp5* and *Rpp14* genes have high N-terminal homology to the tobacco *N* gene and the *L* genes of flax. This finding supports the hypothesis that different specificities have arisen from common ancestral genes (Staskawicz et al. 1995). Also, **the similarities between certain functional motives suggest that resistance genes recognizing different pathogens with strikingly different modes of attack operate through similar, if not identical, pathways.** Parker et al. (1996) isolated several mutations at the *Rpp* loci and could identify with this approach the correct open reading frame.

c) Extracytoplasmic Proteins with a Transmembrane Domain (Group III)

The four tomato genes *Cf-2*, *Cf-4*, *Cf-5* and *Cf-9* active against different races of the fungus *Cladosporium fulvum* form a third group to which also the resistance gene *I2* from tomato active against *Fusarium oxysporum* belongs (De Wit 1995). They are probably **transmembrane proteins containing a small cytoplasmic and a larger extracytoplasmic, glycosilated domain**, and consist primarily of 28 extracytoplasmic LRRs

attached at the C-terminus to the cell by a transmembrane domain and a short cytoplasmic domain. The C-terminal half of the LRR domain of several *C-f* genes shows substantial homology. This conserved domain might interact with the extracytoplasmic domain of another protein to effect signal transduction. Differences between *C-f* genes are mostly confirmed to the N-terminal half of the LRRs, suggesting that **this domain may play a role in the specific recognition** (Jones et al. 1996). Comparisons of the sequence of *Cf-2* and *Cf-9* demonstrated homologies at the C-terminal end containing the transmembrane domain and a part of the extracellular LRR domain responsible for the ligation.

Dixon et al. (1996) proposed two possible models for these two R-genes:

1. The avr-protein might bind at the LRR domain and this complex might react with a membrane-bound NADPH-oxidase which starts the resistance reaction of the plant by producing superoid anions.
2. A complex of R- and avr-proteins might bind a transmembrane kinase which activates in additional reactions that NADPH-oxidase.

Several examples for receptor protein kinases bound in membranes exist in plants, e.g. the RLK5 in *Arabidopsis* (Walker 1993) or the product of the resistance gene *Xa21* of rice (Song et al. 1995).

d) Extracytoplasmic LRRs with a Transmembrane Protein Kinase (Group IV)

In Group IV, the resistance gene *Xa21* causing resistance against the bacterium *Xanthomonas oryzae* combines characteristics of groups I and III and contains an intracellular protein kinase as well as extracellular LRRs, linked via a transmembrane domain. The extracellular part of *Xa21* has homologies to *Cf-9* and *Cf-2*, while the kinase domain is similar to PTO. **These similarities made it probable that for each resistance gene containing a kinase domain, additionally an LRR-protein similar to Cf-9 (protein) is necessary**, while for other LRRs containing resistance genes, e.g. the genes of group II, a protein kinase is available (Bent 1996). Along this line for the protein kinase PTO an LRR-protein (PRF) was found that interacts with PTO and is necessary for the expression of the resistance (Salmeron et al. 1996).

e) The Mlo Powdery Mildew Resistance Gene of Barley (Group V)

A fifth type of the function in inducing resistance is coupled to the powdery mildew resistance gene *mlo* of barley. Mutation-induced recessive alleles (*mlo*) of the barley *Mlo* locus confer a leaf lesion phenotype and

broad spectrum resistance to *Erysiphe graminis* (Büschges et al. 1997). Analysis of mutagene-induced *mlo* alleles revealed mutations leading to alterations of the deduced *Mlo* wild type protein. Susceptible intragenic recombinants isolated from *mlo* heteroallellic crosses show restored *Mlo* wild-type sequences. **The deduced amino acid sequence reveals no homologies to any other described plant resistance gene.** However, significant homologous sequences have been found to rice and *Arabidopsis* (Büschges et al. 1997). This strongly suggests that the Mlo protein is likely to represent a member of a separate protein family and implies a conserved function among plants.

The large protein segment between predicted transmembrane helices is likely to face the cytosol whereas the C-terminal end appears to be located on the extracellular face (Hartman et al. 1989). In addition, a putative nuclear localization sequence motive (NLS) was found, indicating a possible transport of the protein into the nucleus (Nigg et al. 1991). It is not yet clear whether the protein is located in the nuclear membrane. An anticipated frame shift is predicted to shorten the length of the expressed Mlo protein by 75%.

Büschges et al. (1997) assume that this resistance allele represents a complete functional inactivation of the protein whereas the alleles might encode proteins with residual activity. The results show that resistance to *Erysiphe* is caused by a defective *Mlo* gene. For the explanation of the protein action two alternatives exist: (1) Mlo has a negative control function in leaf cell death. It would suppress a default cell suicide programme in foliar tissue; and (2) Mlo has a specific negative regulatory function by down-regulating multiple defence-related function.

Spontaneous cell death in *mlo* genotypes represents the end of an accumulating activation of defence responses. It is concluded that a complete or partial inactivation of the Mlo protein primes or upregulates the responsiveness of the seedling for the onset of pathogen defence (Büschges et al. 1997).

f) Additional Genes

Most resistance genes cloned up till now take part in ligand binding and/or the signal transduction via phosphorylation cascades. To understand the different pathways leading from the identification of the *Avr*-gene product finally to the resistance reaction of the plant, the different components of this system and their interaction have to be elucidated. Up till now only very few additional genes the product of which acts directly or indirectly with the R-gene products have been found. This may be due to two reasons: (1) The signal transduction chains, resulting in resistance, contain only few components; and (2) several proteins

Table 4. Loci required for disease resistance (Altered from Hammond-Kosack and Jones 1996)

Plant	Locus	R-gene	Pathogen	Loss of function	Localization	Reference
Tomato	*Prf*	*Pto/Fen*	*Pseudomonas syringae*	Complete	Linked with *Pto/Fen*	Salmeron et al. (1996)
	Rcr-1, Rcr-2	*Cf-9*	*Cladosporium fulvum*	Partial	Unlinked	Hammond-Kosack et al. (1994)
	Rcr-3, Rcr-5	*Cf-2*		Partial – Complete	Unlinked	Hammond-Kosack and Jones (1996)
Arabidopsis	*Ndr 1*	*Rps2*	*P. syringae pv. toamto*	Complete	Unlinked	Century et al. (1995)
		Rpm1	*P. syringae pv. glycinea*			Century et al. (1995)
		Rpps	*Peronospora parasitica*			Century et al. (1995)
	nim1	*Rpps*	*Peronospora parasitica*	Complete	Unlinked	Delaney et al. (1995)
	Eds1	*Rpps*	*Peronospora parasitica*	Complete	Unlinked	Parker et al. (1996)
Barley	*Rar 1, Rar 2*	*Mla-12*	*Erysiphe graminis*	Nearly complete	2H, unlinked	Freialdenhoven et al. (1996)
	Ror 1, Ror 2	*mlo*	*Erysiphe graminis*	Nearly complete	Unlinked	Büschges et al. (1997)

might be involved, which are not yet found by tagging or their absence is lethal.

Table 4 summarizes genes identified by mutagenesis which are necessary for the function of specific R-genes. In tomato, several genes required for *Cladosporium resistance (Rcr)* were identified in mutageniced homozygous *Cf-9* or *Cf-2* plants. In these mutants, the *Cf* gene function is partially or completely inhibited but there is no linkage (Hammond-Kosack et al. 1994). Another locus, *Ndr1*, the non-race-specific disease resistance against *Pseudomonas*, was detected on chromosome 3 of *Arabidopsis*. **This makes it possible that reactions against fungi and bacteria may rely on identical genes** (Century et al. 1995).

Additional loci required for disease resistance of *Arabidopsis* are *nim1* and *Eds1*, influencing resistance against *Peronospora parasitica* (Delaney et al. 1995; Parker et al. 1996). In barley, loci were identified necessary for the function of powdery mildew resistances. Mutation in *Rar1* and *Rar2* required for *Mla* resistance originally named *Nar1* and *Nar2* reduce the HR production and the induction of disease-relevant gene *Mlat-12*, but not in combination with *Mlg* (Freialdenhoven et al. 1994). The two other loci *Ror1* and *Ror2*, required for *mlo* resistance, inhibit the horizontal *mlo* resistance and the production of papillae (Freialdenhoven et al. 1996). This makes it probable that in barley the resistance against different powdery mildew isolates, based on specific resistance genes, relies on different mechanisms.

Cell death caused by the HR has several similarities with the programmed cell death, apoptosis, observed in mammals and insects (Greenberg et al. 1994). Mutants for cell death have been identified in maize, rice, tomato, barley and *Arabidopsis*. Probably the wild-type genes code for several components of the signal transduction chain resulting in a normal resistance (Jones and Dangl 1996). It is, however, also possible that the mutants cause **unspecific disturbances of the biochemical pathway** (Dietrich et al. 1994).

7. Use of Knowledge Deduced from Genome Analysis in Breeding

The fact that most durable resistances are not coded by a single gene but rather by oligo- or polygenic ones is a reason that under applied aspects marker assisted selection (MAS) **is opening up faster success than the transfer of isolated or even monogenic genes, the function of which is known**. The analysis of QTLs is under rapid development. The whole character will often depend on some major alleles, some of which can be identified. The use of selectable markers will allow a more efficient manipulation of resistance genes during the breeding process. Thus, traits with low heritability can be selected more efficiently. **Regarding the realization of gene pyramiding concepts, MAS can replace extensive virulence tests** (Graner et al. 1995). The use of molecular markers will facilitate the combination of resistance genes which due to the lack of appro-

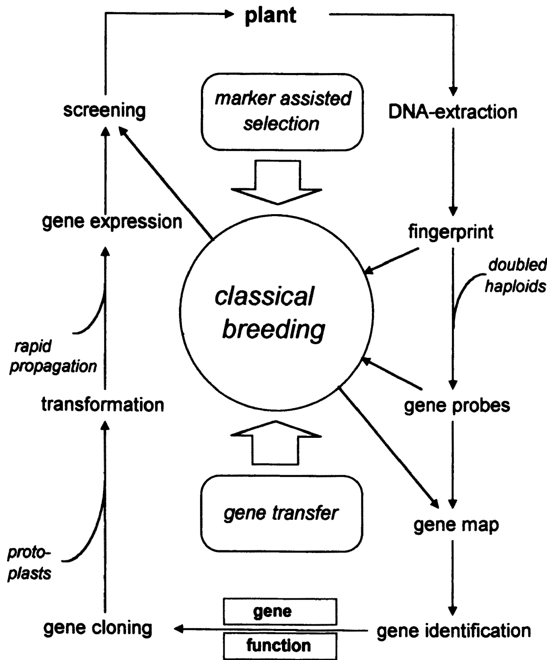

Fig. 1. Connections between different methodological tools: marker assisted selection gene function and gene transfer, together with overlap molecular genetics and breeding steps

priate virulences, cannot be differentiated based on their phenotype. Undoubtedly, molecular markers represent a useful tool for the breeder; their introgression into the breeding process will require, however, a revision of existing selection concepts. Figure 1 combines the MAS with the techniques necessary for gene identification and gene transfer. **The dual purpose of this technique** - for direct use in selection and for helping in gene isolation - is obvious. Fortunately, nature is rather conservative, visible by the high amount of similarity of R-gene sequences. Due to this synteny, it will not be necessary to start gene isolation and function analysis always right from the beginning but to make use of heterologous probes. The *mlo*-gene shows, however, that surprises can be expected.

8. Conclusions

The first phase of genome analysis was mainly characterized by joint efforts to construct comprehensive maps. During the next phase re-

search activities focused on the utilization of these maps for genetic localization of agronomic traits, e.g. by MAS, and for elucidating the function of the responsible genes. With respect to disease resistance, **about 150 alleles of major genes and QTLs have been identified.** There is preliminary evidence that some QTLs might represent less effective alleles of major genes because (1) many QTLs coincide with the genetic position of major genes, and (2) a series of QTL studies revealed one major QTL accounting for most of the genetic variability.

The physical isolation of resistance genes is a prerequisite for the functional analysis of the corresponding genes. Together with verification experiments by transformation, this elucidation will be a central research field. Although presently the most efficient gain of molecular technique is based in the area of MAS, the future will strengthen the transfer approaches. As soon as the gene function is understood, a third development will start, allowing the construction of resistance genes according to their specific needs. Presently only a few data exist on the verification of an anticipated gene function by gene transfer. In most cases, these examples come from the transfer of viral, phagous or bacterial genes. Transferring genes of one higher plant to another higher plant is still rare.

It can be expected that the speed of identifying resistance genes will increase significantly. One possible new strategy is **their identification by using the homology in the gene structure** for its identification. Leister et al. (1997) and Gebhardt et al. (1997) report on the production of R-gene homologues by a PCR approach that uses degenerate primers of conserved domains of NBR- and LRR-type resistance genes. Several primer sets were applied in PCR reactions with templates from rice or barley, genomic DNA or cDNA and tobacco or *Arabidopsis*, respectively. The PCR products were cloned and in both instances clones exhibiting significant homology were isolated. With this new approach **genome-wide mapping data of the rice R-gene homologues revealed several correlations to mapped resistance traits and lesion mimic loci as well as cosegregants of potato R-genes.**

Other approaches make use of the synteny, expecting that the conserved genomes have similar motives active in resistance (e.g. Killian et al. 1995). In most cases, such R-gene candidates have been found since their number was in most cases more than five. The proof which one of the five will be the correct one needs transformation techniques; since this is still difficult to routinely transfer numerous constructs, the answer is still missing. Progress will depend upon the genetic definition of the target gene. Particularly, induced and spontaneous mutants, as well as variants and mapping populations, will be of critical importance.

Uncovering R-genes and their function relies also on good classical genetics and phenotypic characterizations. A fruitful cooperation between classical and molecular genetics is the way to go. All successful crop varieties are selected for disease resistance, but up till now without knowing their exact molecular function. Since this strategy has already been quite successful, it can be expected that after understanding the R-gene

functions, man has for the first time the chance to be more efficient in plant protection than the concurring trial and error approach of pathogens.

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