ORGANIZATION OF GENES CONTROLLING DISEASE RESISTANCE IN THE POTATO GENOME

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Abstract  Nineteen single dominant genes (R genes) for resistance to viruses, nematodes, and fungi have been positioned on the molecular map of potato using DNA markers. Fourteen of those genes are located in five “hotspots” for resistance in the potato genome. Quantitative trait loci (QTL) for resistance to late blight caused by the oomycete Phytophthora infestans, to tuber rot caused by the bacterium Erwinia carotovora ssp. atroseptica, and to root cyst nematodes have been identified on all 12 potato chromosomes. Some QTL for resistance to different pathogens are linked to each other and/or to resistance hotspots. Based on the genetic clustering with R genes, we propose that some QTL for resistance have a molecular basis similar to single R genes. Mapping potato genes with sequence similarity to cloned R genes of other plants and other defense-related genes reveals linkage between candidate genes, R genes, and resistance QTL. To explain the molecular basis of polygenic resistance in potato we propose (a) genes having structural similarity with cloned R genes and (b) genes involved in the defense response. The “candidate gene approach” enables the identification of markers highly useful for marker-assisted selection in potato breeding.

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INTRODUCTION

Short History of Resistance Breeding in Potato

Potato (Solanum tuberosum ssp. tuberosum L.) is the fourth most important food crop worldwide after wheat, maize, and rice (44). Annual production is estimated as ca. 300 million tons. However, potential production could exceed 400 million tons if the diseases that reduce yield by approximately a quarter could be controlled (1). As a clonally propagated crop, potato is vulnerable to pests and diseases affecting leaves, stems, roots, and tubers. Also, after harvest the tuber crop is endangered by fungal and bacterial pathogens, and by insects in hot climates. Infected seed tubers transmit pathogens to the next growing season, thereby causing progressive degeneration of yield.

One of the most devastating famines in Europe, affecting millions of people, occurred in the 1840s. Late blight epidemics caused by the oomycete Phytophthora infestans almost completely destroyed the potato crop (95). Late blight and the various symptoms caused by Potato virus Y (PVY) still pose major disease problems in potato production areas in temperate climates.

At the beginning of the twentieth century, shortly after the rediscovery of Mendel’s laws of inheritance, a tuber bearing wild Solanum species was discovered to be a source of genetic resistance to Phytophthora infestans (94). Based on this discovery, introgression of genes for resistance was initiated by crossing resistant
wild potatoes with susceptible domestic potato varieties. From the 1920s onwards, numerous scientific expeditions to Mexico, Central, and South America, the centers of origin and diversity of the potatoes, have led to the collection and taxonomic description of over 200 wild and 8 cultivated tuber-bearing *Solanum* species (reviewed in 44). When tested with potato pathogens, resistant plants were identified among the many accessions of wild and cultivated potatoes (44, 90). Compared with the huge natural genetic diversity available in the wild relatives of the potato, only a small number of these species have actually been used for introgression of resistance traits into cultivars, because of the introduction of undesirable “wild” traits together with the resistance trait. Several generations of backcrossing and recurrent selection are usually required before acceptable cultivars can be obtained from such materials. Nevertheless, most genes for resistance to viruses, fungi, and nematodes present in modern potato varieties and breeding materials have been introgressed from closely related tuber-bearing *Solanum* species. For further details on the history of resistance breeding in potato the reader is referred to Ross (90) and Hawkes (44).

**Inheritance of Disease Resistance in Potato**

Among the first agronomically relevant traits investigated for mode of inheritance was resistance of potatoes to the wart disease caused by the fungus *Synchytrium endobioticum* (96). The potato (*Solanum tuberosum* ssp. *tuberosum*) is, however, a far from ideal species for genetic analysis: It is tetraploid (2n = 4x = 48) with tetrasomic inheritance and highly heterozygous owing to inbreeding depression after repeated selfing. One to four different alleles are present per locus, resulting in one homozygous and four heterozygous genotypes: homozygous or quadruple (A1A1A1A1), 4-times simplex (A1A2A2A2), duplex/simplex/simplex (A1A1A2A3), duplex/duplex (A1A1A2A2) and simplex/triplex (A1A2A3). Genotypes may combine with each other in crosses resulting in 25 genetic models to be considered in inheritance studies. The simplest genetic model for the inheritance of disease resistance is based on the monogenic inheritance of a dominant resistance allele (*R* gene) present in a tetraploid plant in one of four allelic states, homozygous (*RRRR*) or heterozygous (*RRrr*, *RRrr*, *RRrr*) or (*Rrrr*). The Mendelian ratios expected in offspring of heterozygous resistant and homozygous susceptible plants (*rrrr*) are then (assuming chromosome, not chromatide segregation) 1:0 resistant versus susceptible plants for a *RRrr* parent, 5:1 for a *RRrr* parent, and 1:1 for a *RRrr* parent. One of the most comprehensive studies on inheritance and linkage of single genes for resistance in tetraploid potato was by Cockerham (16) on genes for resistance to *Potato virus X* (PVX) and *Potato virus Y* (PVY).

Flor’s gene-for-gene concept (31) states that for each host resistance (*R*) gene, there is a corresponding pathogen avirulence (*Avr*) gene. The *Avr* gene produces, or results in the production of a ligand that is recognized by the *R* gene product (21). Recognition between these two products is required for elicitation of the
defense responses that are controlled by a wide variety of different genes. The gene-for-gene concept is compatible with most of the single genes for resistance that have been studied in potato species. The concept has been proven by classical genetic analysis, e.g., for the resistance gene \( H1 \) and the corresponding \( Avr \) gene of the root cyst nematode \( Globodera rostochiensis \) (50), and by molecular studies that have identified the PVX coat protein gene as the \( Avr \) gene corresponding to the resistance gene \( Rx \), which is structurally similar to several other \( R \) genes cloned from plants (6).

The prominence of single gene resistance (“vertical”) in the scientific literature has resulted from its simple Mendelian inheritance rather than its prevalence in natural pathosystems. However, non-Mendelian, quantitative variation of resistance levels is frequently observed in wild and cultivated potatoes (11, 90). This “horizontal” or field resistance is assumed to be controlled by polygenes or minor gene complexes. The distinction made between two types of resistance, qualitative and quantitative, vertical and horizontal, controlled by major \( R \) genes and by minor genes or polygenes, is often not clear-cut at the phenotypic level. The relationship between major genes and minor genes for resistance in plant-pathogen interactions may be compared to a few high and prominent mountain peaks standing out in a vast landscape of rolling hills.

**New Tools for Genetic Analysis of Disease Resistance in Potato**

Two technical developments provided the enabling tools for genome-wide studies on qualitative and quantitative disease resistance in potato: the manipulation of the ploidy level and the advent of DNA-based genetic markers. Ploidy reduction from the tetraploid to the diploid level became possible either by pollination of tetraploid \( Solanum tuberosum \) ssp. \( tuberosum \) with certain genotypes of \( Solanum phureja \) \((2n = 2x = 24)\), which induces the parthenogenetic development of diploid female gametes into plants (46, 47), or by regenerating diploid plants from male gametes of tetraploid plants via anther or microspore culture (22, 84). At the diploid level, the complexity of genetic analysis in potato is equivalent to human genetics: Partially heterozygous parents generate segregating offspring, with potato having the advantage that segregating \( F1 \) families with hundreds of sibs can be generated experimentally by crossing parental lines.

DNA-based genetic markers in eukaryotes were first described as restriction fragment length polymorphism (RFLP) between genomic DNA of different strains of yeast (81). The molecular basis of all DNA markers are the point mutations (single nucleotide polymorphism, SNP), insertions, deletions, or inversions of DNA fragments that differentiate the individual genomes of all members of a species. These DNA polymorphisms survived selection during species evolution because they had no negative effect on survival and reproduction. Mendelian inheritance, phenotypic neutrality, and unlimited availability made DNA-based markers important tools for genome analysis (9). Construction of linkage maps based on DNA
markers became feasible with a precision never achieved with classical morphological or isozyme markers.

RFLP markers, in particular, allowed comparative mapping of genomes of sexually incompatible species for the first time. This was possible because the DNA of related species may share high sequence similarity and the RFLP assay is based on nucleic acid hybridization between a labeled marker probe and a membrane-bound genomic target sequence. Hence, depending on the experimental conditions used, cross hybridization is detected between DNA sequences that are not identical but similar, and RFLP markers originating from one species can be used to construct linkage maps in related species. Conserved linkage between loci identified by the same markers in different species indicates not only similarity of DNA sequence but also similarity of genome structure (synteny). A first molecular linkage map of potato was constructed with tomato RFLP markers mapped to the 12 linkage groups of tomato. The study demonstrated for the first time extensive genome collinearity between two closely related plant species (8). Over the past one and a half decades, several linkage maps have been constructed for potato based on RFLP, AFLP (Amplified Fragment Length Polymorphism, 114), SSR (Simple Sequence Repeat), and other PCR-based markers (8, 14, 35–37, 48, 73, 103, 113). Some of those maps can be aligned with the molecular maps of tomato (103) and pepper (65), based on common RFLP markers.

Molecular linkage maps provide the framework for the location of loci for pathogen resistance in the potato genome. Using those maps, genes controlling monogenic and polygenic resistance to various pathogens have been located on the 12 potato chromosomes over the past ten years. Mapping experiments carried out in different laboratories have used different, generally diploid, potato genotypes. Independent maps can be aligned provided some common potato or tomato RFLP markers are used for their construction. RFLP anchor markers (Table 1) allow positional information to be integrated into a single potato function map for resistance (Figure 1). This map is an interpretation of various mapping experiments and summarizes our current knowledge of the organization of genes for resistance in the potato genome. Using anchor markers, the potato function map for resistance can also be linked to syntenic regions in the related genomes of tomato, tobacco, and pepper.

**MAP POSITIONS OF POTATO GENES FOR RESISTANCE TO NEMATODES**

The nematode species most damaging to the cultivated potato are the root cyst nematodes *Globodera rostochiensis* and *Globodera pallida*, followed by root knot nematode species of the genus *Meloidogyne* (82). Since 1948 (26), genes for resistance to *G. rostochiensis* and *G. pallida* have been discovered in a range of wild potato species. A few genes have been introgressed in breeding lines and cultivars (reviewed in 82, 90).
**TABLE 1**  
*R* genes and QTL mapped in potato

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Gene</th>
<th>Anchor markers</th>
<th>Pathogen</th>
<th>Reference</th>
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<td><em>Eca</em> QTL</td>
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<tr>
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<td><em>R2</em></td>
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<td><em>G. pallida</em></td>
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<td><em>P. infestans</em></td>
<td>78, 106</td>
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<td>Gbssl</td>
<td><em>E. carotovora</em> ssp. <em>atroseptica</em></td>
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</table>
The positions of loci for nematode resistance identified to date in potato are shown on the function map for resistance (Figure 1, Table 1). The first nematode-resistance gene mapped to the potato molecular map using RFLP markers was the monogenic dominant \( Gro1 \) gene for resistance to \( G. rostochiensis \), which is located on chromosome VII (4). This gene originated from an interspecific hybrid
(2n = 2x = 24) between S. tuberosum and the wild potato species S. spegazzinii and conferred resistance to all G. rostochiensis pathotypes tested. Gro1 may be identical to the Fb gene that was identified by Ross (89) in S. spegazzinii (syn. S. famatinae).

H1, a dominant gene that confers durable resistance to pathotype Ro1 of G. rostochiensis, was discovered nearly 50 years ago in the cultivated potato species S. tuberosum ssp. andigena (27, 105) and was most widely used in potato breeding for nematode resistance. The H1 locus has been mapped with RFLP markers to the same genomic segment of potato chromosome V in two different diploid populations (34, 83). GroV1 for resistance to G. rostochiensis originating from the wild potato species S. vernei (49). Based on pedigree information, accession CPC 1673 of S. tuberosum ssp. andigena was the source not only of the H1 gene but also of Gpa2, a major gene for resistance to G. pallida. The Gpa2 gene was localized with AFLP and RFLP markers on potato chromosome XII (93).

Only one resistance locus against Meloidogyne species has been identified so far in the potato genome. The gene Rmch1 for resistance to M. chitwoodi was introgressed into cultivated potato from the wild potato species S. bulbocastanum by somatic hybridization and is located on chromosome XI (13).

A number of factors for quantitative resistance (Quantitative Trait Loci, QTL) to root cyst nematodes have also been identified and mapped. QTL Gro1.2, Gro1.3, and Gro1.4 for resistance to G. rostochiensis were localized with RFLP markers on potato chromosomes X, XI, and III, respectively. The source of resistance was S. spegazzinii (56, 58). The same S. spegazzinii parent also carried quantitative resistance to G. pallida. QTL mapping of this resistance resulted in one major QTL Gpa on chromosome V (57). Two further QTL for resistance to G. pallida, Gpa5 and Gpa6, mapped to chromosomes V and IX, respectively (92). Gpa5 is tightly linked to Gpa. Grp1, a QTL for broad-spectrum resistance to both cyst nematode species G. rostochiensis and G. pallida, was independently mapped to the same segment on chromosome V as QTLs Gpa and Gpa5 (91, 92). Grp1, Gpa, and Gpa5 originate from different genetic backgrounds involving several wild potato species. QTL mapping of resistance to G. pallida in a tetraploid cross using AFLP and SSR markers allocated a QTL (Gpa4) to chromosome IV, which was possible via the linkage of Gpa4 to SSR marker Sm3016 with a known map position (10).

In contrast to quantitative resistance against Phytophthora infestans or Erwinia carotovora spp. atroseptica, which are controlled by factors on every potato chromosome (see below), quantitative resistance to nematodes seems to be controlled mainly by few QTL with large effects.

MAP POSITIONS OF POTATO GENES FOR RESISTANCE TO VIRUSES

At least 40 viruses are known to infect potatoes in the field (51). The most important viruses for potato cultivation are Potato leaf roll virus (PLRV) and Potato virus Y (PVY) (90), followed by Potato virus X (PVX), A (PVA), M (PVM), S (PVS) (19),
and *Potato mop-top virus* (PMTV) (99). Most potato viruses are transmitted by aphids, except PMTV, which is transmitted by the zoospores of the powdery scab pathogen *Spongospora subterranea* (a protist) and PVX, which has no vector but is contact-transmitted. Viruses cannot be chemically controlled in the field, and the control of virus vectors with pesticides does not efficiently control the spread of viruses in the field in most cases. Therefore, virus-free seed potatoes and resistant cultivars form the basis for virus control in potato.

Single dominant genes for resistance to viruses have been discovered and genetically characterized in wild and cultivated potato species since the middle of the previous century (5, 16, 90, 102). The common types of single gene resistance to viruses in potato are extreme resistance (ER) and hypersensitive resistance (HR). The genes for ER confer high levels of resistance to all or most virus strains. Extremely low amounts of virus are detected in inoculated plants and no symptoms are observed (3, 16, 90). The genes for hypersensitive resistance (HR) are usually virus strain group specific. Their phenotypic expression is characterized by development of necrotic symptoms in the virus-infected tissues (16, 52), whereas such symptoms are very limited and usually absent in plants expressing ER (3, 42, 90). The nomenclature of virus resistance genes in potato has been reviewed (109).

The positions of loci for virus resistance known to date in potato are shown on the function map for resistance (Figure 1) (Table 1). Genes *Rx1* and *Rx2* confer ER to *Potato virus X* (PVX) in *S. tuberosum* ssp. *andigena* (16) and *S. acaule* (102), respectively. *Rx1* and *Rx2* have been introgressed into potato breeding lines and mapped to chromosomes XII and V, respectively (87). The gene *Nb* conferring HR to PVX has been mapped to the same region on chromosome V as *Rx2* (20). *N*<sub>xphu</sub> controls HR to PVX in *S. phureja*, a diploid cultivated species, and resides on the south arm of chromosome IX (104).

On chromosome XI, the same region contains genes *Ry<sub>adv</sub>* and *Ry<sub>sto</sub>* for ER to *Potato virus Y* (PVY) (12, 41, 42) and *Na<sub>adv</sub>* for HR to *Potato virus A* (PVA) (40). *Ry<sub>adv</sub>* and *Na<sub>adv</sub>* (40–42) originate from an accession of *S. tuberosum* subsp. *andigena* (75, 111), whereas *Ry<sub>sto</sub>* is thought to be derived from *S. stoloniferum* (12), although pedigree information might not be reliable in this case. Genes for ER or HR to PVY, PVA, and *Potato virus V* with a probable common origin in *S. stoloniferum* cosegregate, which suggests that all these genes might reside on chromosome XI (3, 108). This suggestion remains to be tested with molecular markers.

The gene *Ns* for resistance to *Potato virus S* (PVS) was tagged by RAPD markers of unknown position in the potato genome (67). No genes for resistance to the *Potato leaf roll virus*, *Potato virus M*, and *Potato mop-top virus* have been mapped to date. No QTL mapping for quantitative virus resistance has been reported in potato.

MAP POSITIONS OF POTATO GENES FOR RESISTANCE TO FUNGI

Late blight, the most important so-called fungal disease of potato (53, 107), is caused by the oomyceteous fungus *Phytophthora infestans*, which is, in fact, more closely related to brown algae than to higher fungi (107). Intentional breeding for
resistance to late blight began nearly 100 years ago with the introgression of resistance from *S. demissum*, a wild potato species indigenous to Mexico (see 116 for review). On one hand, resistance to late blight is determined by dominant *R* genes inducing an HR response upon infection with specific races of *P. infestans*. Eleven such *R* genes have been described (66). On the other hand, quantitative, race-nonspecific resistance controlled by an unknown number of genes is also known.

The *R1* locus for resistance to late blight was identified on potato chromosome V by RFLP mapping (60). The genes *R3*, *R6*, and *R7* with different pathogen race specificities have been located on chromosome XI in the same genome segment (23, 24). *R2* has been mapped with AFLP markers to chromosome IV (64). All these *R* genes originate from the wild potato species *S. demissum*. Recently, late blight-resistance genes *Rber* of *S. berthaultii* and *Rblc* of *S. bulbocastanum* were identified and mapped to chromosomes X and VIII, respectively (30, 76). These genes confer resistance to contemporary *P. infestans* races carrying multiple virulence factors.

Genetic dissection of quantitative resistance to *P. infestans* using DNA-based markers has been done in several diploid mapping populations (17, 30, 38, 61, 78, 97) and in one tetraploid mapping population (69). Factors controlling quantitative resistance to *P. infestans* have been found on almost every potato chromosome (Figure 1) (Table 1), confirming the truly polygenic nature of this trait. Linkage to anchor markers uncovered some QTL in similar regions of the potato genome in different mapping populations. In this respect, the most promising QTL for marker-assisted breeding are those on chromosomes III (30, 61, 78), IV (61, 72, 78, 97), V (61, 78, 97), and VI (17, 61, 78). The most significant and most reproducible QTL for foliage and tuber resistance to *P. infestans* is located on chromosome V close to the anchor marker *GP179*. This QTL and a second one linked to the marker *GP76* on chromosome VI (Figure 1) are particularly interesting because both are linked with QTL for plant maturity (17, 78). Breeding for field resistance to late blight under long day conditions (e.g., in Central and Northern Europe) usually results in late-maturing cultivars (116). The same correlation is observed at the two QTL on chromosomes V and VI: Plants having alleles that increase resistance mature later and vice versa. Therefore, resistance to late blight and delayed plant maturity may be pleiotropic effects of the same gene(s).

Only one gene for resistance to a fungus other than *P. infestans* has been identified and mapped in potato. The *Sen1* gene of unknown origin has been located on potato chromosome XI (Figure 1) and confers resistance to potato wart, a quarantine disease caused by *Synchytrium endobioticum* that destroys the tubers (45).

### MAP POSITIONS OF POTATO GENES FOR RESISTANCE TO BACTERIA

The two most important bacterial diseases of cultivated potato, blackleg of stems and tuber soft rot, are caused by *Erwinia* species. No source of monogenic resistance is known (29). Quantitative trait loci for tuber and foliar resistance to *Erwinia carotovora ssp. atroseptica* (*Eca*) have been mapped in diploid hybrids originating...
from intercrossing *S. tuberosum* with the wild potato species *S. chacoense* and *S. yungasense* (119). For one F1 hybrid population, a linkage map was constructed based on AFLP and RFLP markers, including three resistance gene-like markers (see below). The QTL analysis revealed complex inheritance of resistance to *Eca*. Loci for genetic factors affecting resistance to *Eca* were found on all 12 potato chromosomes. Most QTL can be incorporated in the potato function map for resistance (Figure 1) using RFLP anchor markers. The QTL with the largest and most reproducible effect on tuber resistance mapped to chromosome I.

**CONCLUSIONS FROM THE LOCATION OF POTATO GENES FOR RESISTANCE**

As known from classical genetic studies in plants, single genes conferring resistance to specific races of a pathogen may be tightly linked, either because they are multiple alleles of one gene or because several related genes with similar function are located next to each other in the same narrow genome segment (85). Such clustered gene families evolve from common ancestors by local gene duplications followed by structural and functional diversification. Several clusters of this type have now been analyzed at the molecular level in plants, e.g. in flax (2), tomato (80), or lettuce (70).

Clusters of R Genes

In potato, DNA markers made possible the identification of complex loci having several *R* genes. At least three *R* genes (*R3*, *R6*, *R7*) for race-specific resistance to *P. infestans* are organized in a cluster in the distal segment of chromosome XI anchored by the marker locus *TG105* (a) (Figure 1). At least two genes (*H1*, *GroVI*) for resistance to the cyst nematode *G. rostochiensis* in two different potato species identify another cluster on chromosome V.

More surprisingly, genes for resistance to different pathogens also form clusters. This was first observed in the potato genome when the *R1* gene for resistance to *P. infestans* and the *Rx2* gene for resistance to PVX were found to be closely linked to the same RFLP anchor marker *GP21* on chromosome V (60, 87). Later, *Nb* controlling HR to PVX was mapped to the same *R* gene cluster (20). Two additional clusters of *R* genes with different pathogen specificities are known (Figure 1): Genes *Rxadj*, *Naadj* (both virus-resistance genes), *Rmc1* (nematode-resistance gene) and *Sen1* (resistance to the wart fungus) form a cluster on chromosome XI, anchored by marker *CP58*. Genes *RxI* (virus-resistance gene) and *Gpa2* (nematode-resistance gene) are clustered on chromosome XII close to anchor marker *GP34* (Figure 1) (Table 1).

**Linkage Between QTL and R Gene Loci**

QTL are positioned on the molecular map with less precision than single genes because individual recombinants between a QTL and a linked Mendelian marker
cannot be identified. The effects of quantitative trait alleles can be detected with markers that span a genetic distance of a few centimorgans up to a whole linkage group that may be tens of centimorgans. The phenotypic effect detectable with a marker decreases with increasing genetic distance between the marker and the QTL. The genetic distance covered by a QTL correlates with the size of the effect, not with the number of genes causing the effect. A single gene with a large effect is still detectable by relatively distant markers, whereas a gene or a group of genes with small effects is detectable only by the most closely linked markers. Independent QTL maps can be compared using as anchors single markers or marker intervals with the most significant trait effects. Integration of QTL for resistance to *P. infestans*, cyst nematodes, and *Erwinia carotovora* ssp. *atroseptica* in the potato function map for resistance reveals several examples of linkage between QTL and *R* genes. This was first observed when the RFLP markers *GP2I* and *GP179* flanking the *R1* gene within a 3-cM interval also detected the largest effects on quantitative resistance to *P. infestans* on chromosome V (61). The most conspicuous genetic hotspots containing multiple genes for *R* gene resistance and quantitative resistance to different pathogens are located on chromosomes V, XI, and XII of potato (Figure 1).

### Comparison of Function Maps for Resistance Between Solanaceous Species

The potato function map for resistance can be anchored with markers to colinear segments of the related genomes of tomato, tobacco, and pepper, revealing synteny between resistance loci of these species. However, structural synteny does not necessarily implicate functional synteny. Only four selected and particularly interesting cases of synteny between resistance loci are mentioned here. For more detailed comparisons among Solanaceae genomes the reader is referred to Leister et al (59) and Grube et al (39).

1. A map segment on the short arm of potato chromosome VI (marked by *GP79*; Figure 1) having QTL for resistance to both *P. infestans* and *Eca* (78, 119) is syntenic with a resistance hotspot on tomato chromosome 6, including genes for resistance to a nematode (*Mi*), an aphid (*Meu1*), a virus (*Ty1*), and fungi (*Cf2, Cf5, Ol-1*) (59).

2. Gene *Hero* for resistance to the nematode *G. rostochiensis* maps to a similar position on tomato chromosome 4 (33) as QTL for resistance to *P. infestans* (61, 78) and *G. pallida* (10) on the collinear potato chromosome IV.

3. Marker *CP58* anchors the resistance hotspot to viruses (*Ry*adg, *Na*adg), a nematode (*RMc1*), and a fungus (*Sen1*) on the North arm of potato chromosome XI to the gene *N* for resistance to *Tobacco mosaic virus* (TMV) in tobacco (45).

4. The resistance gene cluster on the South arm of potato chromosome XI is linked by markers *TG105* and *TG36* to *I2* for resistance to the *Fusarium*
oxysporum in tomato (98) and L for resistance to TMV in pepper (62), respectively.

Clustering of genes controlling monogenic and polygenic resistance to diverse pathogens, as observed in the potato genome, may occur by chance alone or may be a consequence of reduced recombination fractions due to proximity of the centromere. Alternatively, the clustering suggests an underlying principle similar to the one known for clustered genes for resistance to specific races of a single pathogen. The first implication of this principle is that monogenic resistance to viruses, nematodes, and fungi may be encoded by alleles of a single gene or by paralogous members of the same gene family. The latter option is now well supported by molecular data (see next section). The second implication is that some QTL for quantitative resistance may be structurally related to R genes acting against the same or a different pathogen. The third implication is that linked QTL for resistance to different pathogens, such as P. infestans, Eca, and cyst nematodes, may be similar at the molecular level.

MOLECULAR BASIS FOR MONOGENIC AND POLYGENIC RESISTANCE IN POTATO

R Genes Cloned from Potato

Only during the past decade have plant genes controlling monogenic resistance been cloned and characterized (reviewed in 28, 43, 118). R genes of unrelated plant species that confer a gene-for-gene type of resistance to viruses, bacteria, fungi, or nematodes possess structural similarities, allowing their classification in four major structural groups regardless of pathogen specificity. Most of the R genes characterized to date belong to a superfamily of genes sharing a putative nucleotide binding site (NBS) or CheY domain (receiver domain common to many proteins of His-Asp phosphotransfer pathways) (86) and a leucine-rich repeat (LRR) domain (NBS/CheY-LRR genes). The second group are resistance genes that have only an LRR domain (LRR genes) in common. Members of the third group share an LRR and a protein kinase domain (PK-LRR genes), and members of the fourth group are protein kinases without a LRR domain (PK-type genes). Proteins with such domains may function as receptors or downstream components of signal transduction pathways leading to the transcriptional activation of a battery of “signal response” genes (68). In the case of R genes, the responding genes are those required for various defense reactions that eventually prevent advancement of pathogen infection.

Three R genes have been cloned from potato. Two of these genes, namely Rx1 and Gpa2 (6, 112), reside in the resistance hotspot on chromosome XII (Figure 1). Rx2 from the resistance hotspot on chromosome V was cloned based on sequence similarity with Rx1 (7). The three genes are members of the NBS/CheY-LRR superfamily (see DC Baulcombe, this volume), similar to the tobacco gene N
(117) and the genes I2 (79, 100) and Mi (74) of tomato. As mentioned above, the map positions of these R genes of tobacco and tomato are syntenic with resistance gene clusters on potato chromosomes XI and VI (Figure 1).

**Linkage Between R Genes, Resistance QTL, and Resistance Gene-Like Loci in Potato**

Some short sequence motifs are highly conserved between R genes of the NBS/CheY-LRR class. Primers designed to such motifs have been used to obtain, by the polymerase chain reaction (PCR), potato gene fragments that, as expected, have DNA sequence similarity with known R genes (59). RFLP mapping of these resistance gene-like (RGL) markers identifies at least 20 RGL loci on the potato molecular map. Most potato RGL probes detect complex gene families with some members tightly linked and others mapping to different loci (59, 119; C Gebhardt, unpublished results). Assuming 20 RGL loci, each having between five and ten copies of NBS/CheY-LRR genes, it is estimated that the potato genome contains at least 100–200 genes of this class. In Arabidopsis, NBS/CheY-LRR genes make up 1% of the genome (71). Moreover, gene families homologous to the tomato Cf genes for resistance to the fungus Cladosporium fulvum (LRR genes) and the tomato Pto gene (a protein kinase) have been mapped to syntenic segments of the potato genome (59). Therefore, the RGL loci discovered so far in the potato genome may represent just the tip of the iceberg. Integrating RGL loci into the potato function map for resistance reveals tight linkage between RGL loci and loci controlling monogenic and/or polygenic disease resistance (Figure 1) (59, 119). Molecular characterization of the Gro1 and R1 loci (C Gebhardt, unpublished data) and the Ry locus (J Valkonen, unpublished data; DC Baulcombe, personal communication) has revealed clusters of genes with sequence similarity to known R genes.

Based on current molecular evidence, we expect that most, if not all, of the single dominant genes for resistance that populate the potato function map are primarily encoded by NBS/CheY-LRR genes or one of the other major classes of resistance genes, irrespective of their pathogen specificity. We propose that not only monogenic resistance but also genetic factors controlling quantitative resistance are encoded by the same classes of genes (61, 78, 91, 119).

**Linkage Between Resistance QTL and Defense-Related Genes**

Genes involved in defense reactions against pathogens (55) were identified, characterized, and cloned long before the first R genes. In potato, many of them are organized in clustered gene families (C Gebhardt, unpublished data). Defense genes have been mapped or tested for linkage with QTL for resistance in potato (37, 61, 106). Integrating defense-related loci in the potato function map reveals linkage with QTL for resistance to P. infestans and/or Eca (Figure 1). Genes involved in defense responses are candidates, therefore, for controlling quantitative resistance. The most conspicuous candidates based on map position are:
1. Genes encoding phenylalanine ammonia lyase (PAL, linkage groups III, IX, X) and 4-coumarate:CoA ligase (4CL, linkage group III), both of which are key enzymes in the phenylpropanoid metabolism leading to the production of phytoalexins (32).

2. Pathogenesis-related (PR) genes encoding glutathione-S transferases (prp1, linkage group IX), acidic and basic glucanases (GluA, GluB, linkage group I), lipoxygenases (Loc, linkage group VIII), proteinase inhibitors (Pin, PinI, linkage groups III and IX), osmotins (Osm, linkage group VIII), and loci detected by tobacco RFLP probes for PR-1a and PR-5 (115) (NtPR-1a, NtPR-5, linkage groups X and XII).

Models to Explain the Outcome of R Gene–Mediated and Quantitative Resistance

How could quantitative resistance to pathogens be explained based on genes that have a receptor-like structure similar to R genes or that are members of defense gene families?

A major QTL such as Grp1 that is located in the resistance hotspot on potato chromosome V and explains 45% to 77% of the phenotypic variance (91, 92) might be, in fact, a single gene. The Mendelian segregation might just be difficult to score precisely because of the effects of environmental factors on the phenotype. Environmental effects on resistance expression have been widely documented in plants, including potato (88, 90).

Only the extremes of multiple alleles at a resistance locus may express full resistance or full susceptibility, whereas other alleles may have an intermediate effect on resistance. At the molecular level, allelic variation in cis-regulatory regions for transcription or translation of an R gene or a defense gene could modulate the cellular concentration of the protein in different tissues at different developmental stages, thereby quantitatively affecting the kinetics of signal transduction and defense response. For example, the same defense genes are activated in potato irrespective of whether or not the interaction with P. infestans leads to resistance or susceptibility. The difference between the outcome of the interaction is the induction kinetics of the defense response (32). Quantitative effects may also result from allelic variation in the structural part of a gene, which may modify in a nondisruptive way the binding affinities for interacting molecules such as an Avr gene product or proteins of the signal transduction pathway.

The biologically active plant receptor that interacts with the avirulence factor may be a dimeric protein or a protein complex of even higher order. Hence, binding specificity for the avirulence factor, efficiency, and kinetics of signal transduction and defense reaction would then be the result of interaction of two or several different NBS/CheY-LRR subunits. As all plants seem to have numerous genes of this type, countless possibilities are available for genotype-dependent quantitative variation of resistance phenotype. Monogenic resistance would then be
nothing but the outcome of a perfect match between the product of a recognized gene for resistance and the products of other members of this ubiquitous gene family.

The oligomer receptor model combined with allelic variability at $R$ loci and at loci involved in signal transduction and defense responses is particularly attractive because it provides a plausible, although not exclusive, explanation for several reported phenomena:

1. The influence of the genetic background on resistance phenotype. Modification or even suppression of the resistance phenotype is observed in the offspring, depending on which susceptible parent the resistance source is crossed with (15, 25, 40, 90, 110). $R$ gene specificities different from those present in the parents may appear in hybrid progeny (25, 94). In offspring segregating for quantitative resistance, resistance levels higher or lower than those present in the parents may be expressed (transgressive segregation) (60, 90).

2. Epistatic interactions between resistance QTL (30, 63, 92).

3. Difficulty of proving a physical interaction between an $R$ gene product and an $Avr$ gene product (28).

CANDIDATE GENES FOR MARKER-ASSISTED RESISTANCE BREEDING

The “Know Where” of $R$ genes, QTL, and hotspots for resistance on the potato molecular maps provides the “Know How” for marker-assisted combination of various genes for resistance. The positional information also gives access to numerous, potentially useful DNA markers (14, 37, 73, 103) beyond those developed within a particular mapping experiment. Crossing programs have been started using as parents the same genotypes that were the source of resistance in mapping experiments. Tightly linked DNA markers (54, 77; C Gebhardt, unpublished data) are being used to select plants at the seedling stage that have combinations of genes for resistance to cyst nematodes, PVY, PVX, and potato wart (C Gebhardt & J Valkonen, unpublished data). In this case, the marker genotype predicts the resistance phenotype because we do know which marker allele (e.g., a PCR product of specific size) is linked in coupling phase with the resistance gene (known marker phase) and is, therefore, coinherited with the resistance gene, except when recombination has occurred (linkage disequilibrium between DNA marker and resistance trait).

Breeding potato cultivars is, however, based on intercrossing hundreds of genotypes of various pedigrees. In such a wide gene pool, prediction of resistance phenotype based on marker genotype is not straightforward because, owing to multiple generations of meiotic recombination separating the individuals in such a gene pool, linkage equilibrium may have been reached between a specific gene
for resistance and an even closely linked marker (unknown marker phase); in other words, a singular marker allele known to be coinherited with resistance in a single cross may not be coinherited with that same resistance in a wide gene pool (77). Only when the marker resides within the resistance gene itself, or is physically close to the resistance gene, is recombination between marker allele and resistance absent or rare even after many generations of meiotic recombination. In this case, linkage disequilibrium between marker allele and resistance gene is still strong, even in wide gene pools (18). Markers based on DNA polymorphism of the genes controlling monogenic or quantitative resistance will be, therefore, the ultimate diagnostic tools for marker-assisted resistance breeding.

Markers have been found that are diagnostic for the presence of the $R_yadg$ gene for resistance to PVY in a wide potato gene pool (54, 101). These markers are based on a resistance gene-like sequence that maps to the resistance hotspot including $R_y$ on potato chromosome XI (59). Moreover, markers from within the resistance hotspot on chromosome V that includes the $R_I$ gene are associated with QTL for late blight resistance and plant maturity when scored in more than 400 potato cultivars (C Gebhardt, unpublished data). Thus, candidate gene markers are excellent tools when searching for “universal” markers for marker assisted selection by linkage disequilibrium mapping in wide gene pools. On the other hand, the finding of linkage disequilibrium between a candidate gene marker and an $R$ gene or QTL for resistance provides excellent support for the hypothesis that the candidate gene is indeed the resistance gene or at least is located physically very close to the resistance gene.

OUTLOOK

The challenge for the future is the molecular identification of those genes that are important for controlling quantitative disease resistance. Quantitative resistance is difficult to manipulate by phenotypic selection in breeding programs. Significant progress may be achieved when we learn how to improve field resistance to disease by combining superior alleles of known genes for quantitative resistance. The potato function map for resistance in combination with candidate gene maps, the analysis of sequence variation present in candidate gene loci, and the association of such variation with resistance phenotype in wide gene pools are considered to be promising approaches to reach that goal in tetraploid potato.

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Figure 1  (from previous page) Potato function map for pathogen resistance. Twelve linkage groups corresponding to the 12 potato chromosomes are shown schematically with approximate genetic distances. Relative positions of potato/tomato anchor markers are indicated in black to the left of the linkage groups. Mapped R genes and QTL for resistance to different pathogens of potato (solid letters) or tomato, tobacco and pepper (outlined letters) are shown at their approximate map positions relative to anchor markers. Different colors indicate the pathogen type. Green: genes for resistance to fungi. Red: genes for resistance to nematodes and aphids. Blue: genes for resistance to viruses. Map segments having QTL for resistance to P. infestans and E. carotovora ssp. atroseptica are marked as green and violet rectangles, respectively. Loci mapped with resistance gene-like markers of potato (St and RGL loci) are indicated in purple to the right of the linkage groups. Loci detected by pathogenesis related and defense gene markers of potato or tobacco (Nr loci) are shown in blue to the right. Small letters in parenthesis indicate that the same marker probe identifies more than one locus. For further details the reader is referred to Table 1 and References 37, 59, 78, 119, 106.