Genetic architecture of basal resistance of barley to *Puccinia hordei*

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Abstract

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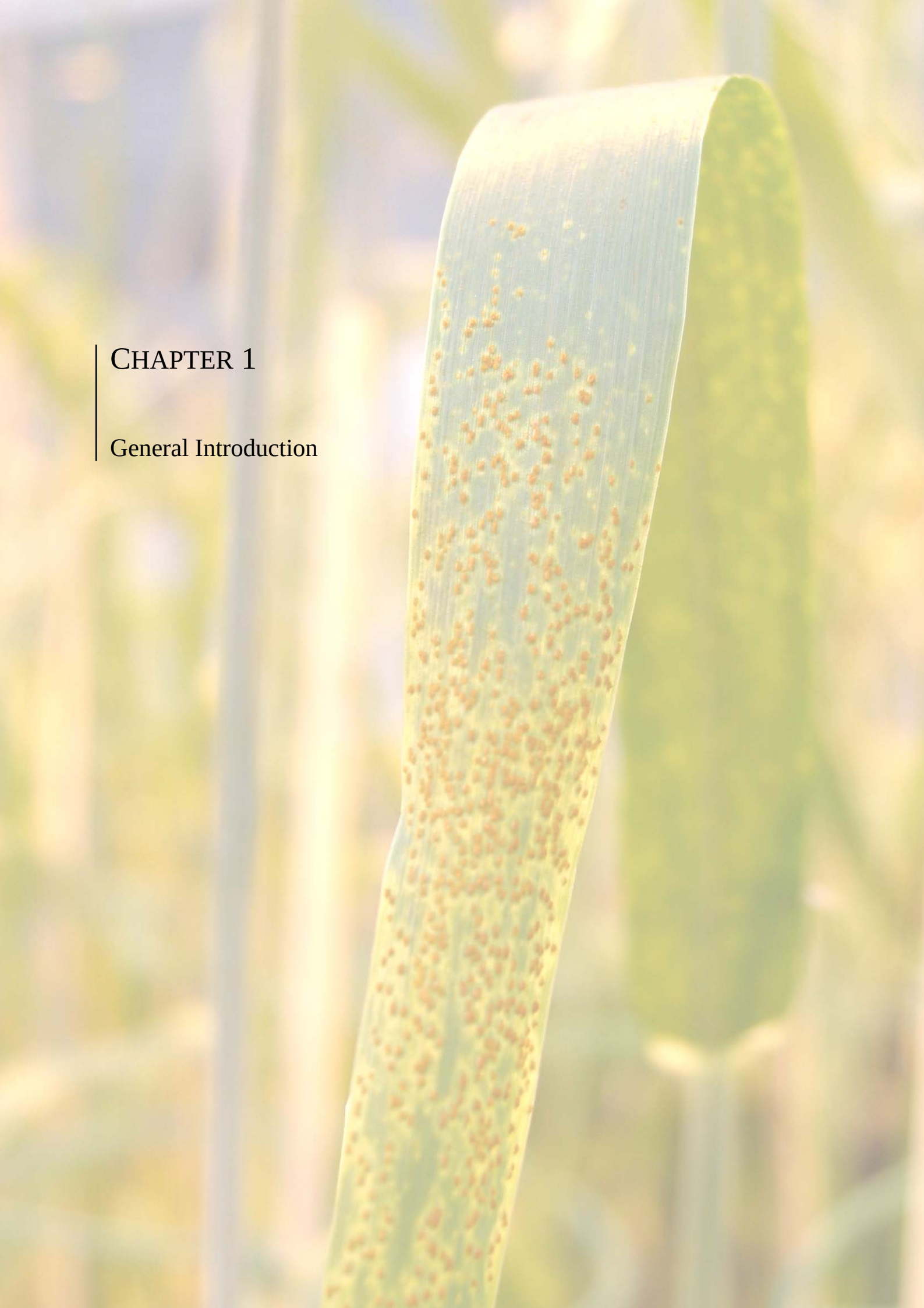
Partial resistance to leaf rust (*Puccinia hordei* Otth) in barley is a quantitative resistance that is not based on hypersensitivity. This resistance hampers haustorium formation and results in a long latency period in greenhouse tests. The resistance is due to genes with relatively small, quantitative effects, located on so called quantitative trait loci (QTL). A detailed chromosome map of barley, containing 3,258 molecular markers, was constructed and used as a platform to compare the genetic positions of QTLs across different mapping populations. This confirmed that partial resistance in barley to *P. hordei* is controlled by a high diversity of genes, each mapping population segregating for a different set of QTLs. Another consensus map was constructed that gathered together 775 barley microsatellite markers. The introgression of single QTL-allele or combination of QTL-alleles in near isogenic lines (NILs) allowed us to confirm the effect of three target QTLs (*Rphq2*, *Rphq3* and *Rphq4*) in seedling disease tests performed in greenhouse compartments and in field disease tests. The use of several leaf rust isolates revealed the clear isolate-specific effect of *Rphq4*. Gene *Rphq2* was easy to detect in seedlings of the corresponding NILs and was located in a physical region of high recombination. The position of *Rphq2* was refined to a 0.1 cM genetic interval that corresponds probably with only a relatively short stretch of DNA. This makes it feasible to pick the gene up from a bacterial DNA (BAC) library in not too distant future.

Contents



CHAPTER 1

General Introduction

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Barley leaf infected with leaf rust

General introduction

Rusts and barley leaf rust

Rust fungi (Basidiomycota, Uredinales) consist of more than 5,000 species of obligate plant pathogens that possess some of the most complex life cycles in the Eumycota (Hiratsuka and Sato 1982). Rust diseases cause serious economic damage worldwide on agricultural, forest and ornamental plants. These fungi are of great interest not only for the economic problems that they cause, but also for their highly specialised relationship with host plants. One of the unique features of rust fungi is that they have up to five functionally and morphologically different spore states in their life cycles. The typical progression of spore states is basidiospore (*N*), pycniospores or spermatium (*N*), aeciospore (*N*+*N*), urediospore (*N*+*N*), teliospore (*N*+*N* → 2*N* → *N*). This is further complicated because, in addition to the different numbers of spore states, they often need two unrelated groups of host plant species to complete their life cycles (heteroecious). Some can complete their life cycles on only one kind of host plant (autoecious). For many rust species, like *Puccinia hordei*, completion of the sexual stages is not required for perpetuation of the rust in nature, since the asexual stage (uredia) may continue infinitely.

Leaf rust is an important disease of barley (*Hordeum vulgare* L.) in many regions of the world. Yield losses up to 32% have been reported in susceptible cultivars. The causal agent, *Puccinia hordei* Otth, is a heteroecious fungus with the dikaryotic stage limited in nature to *H. vulgare* and the sexual stage to *Ornithogalum* species. Parasitically the fungus is confined to the source host species, except that reciprocal inoculations with leaf rust of *H. spontaneum* and *H. vulgare* L. were successful (Anikster and Wahl 1979). Telia on the main host are profusely formed where *Ornithogalum* plants are present. D'Oliveira (1960) demonstrated that 32 species of *Ornithogalum* are compatible with *P. hordei*. The center of origin and diversification of these species coincides at least partly with that of *H. spontaneum* - the putative progenitor of cultivated barley - but includes the regions where barley has been cultivated since remote antiquity.

To initiate the dikaryotic stage, the urediospore forms a germ tube that responds to topographical features of the leaf surface so that it grows towards a stoma and recognises its presence by responding to the ridges around the stomatal lips (Heath et al. 1997; Vaz Patto and Niks 2001). In response to the stomatal lips, the fungus sequentially forms an appressorium over the stomatal opening, an infection peg that grows between the guard cells, a torpedo shaped substomatal vesicle in the substomatal space, and an infection hypha that grows intercellularly between mesophyll cells (Fig. 1). Then, the fungus forms an intercellular mycelium from which intracellular haustoria are formed. These haustoria are generally

considered to be feeding structures and they develop from haustorial mother cells that adhere to the plant cell surface (Mendgen et al. 2000). During the other, monokaryotic, parasitic stage, rust fungi usually penetrate directly into epidermal cells and the only pre-penetration structure is the appressorium produced by the basidiospore germ tube (Longo et al. 2006).

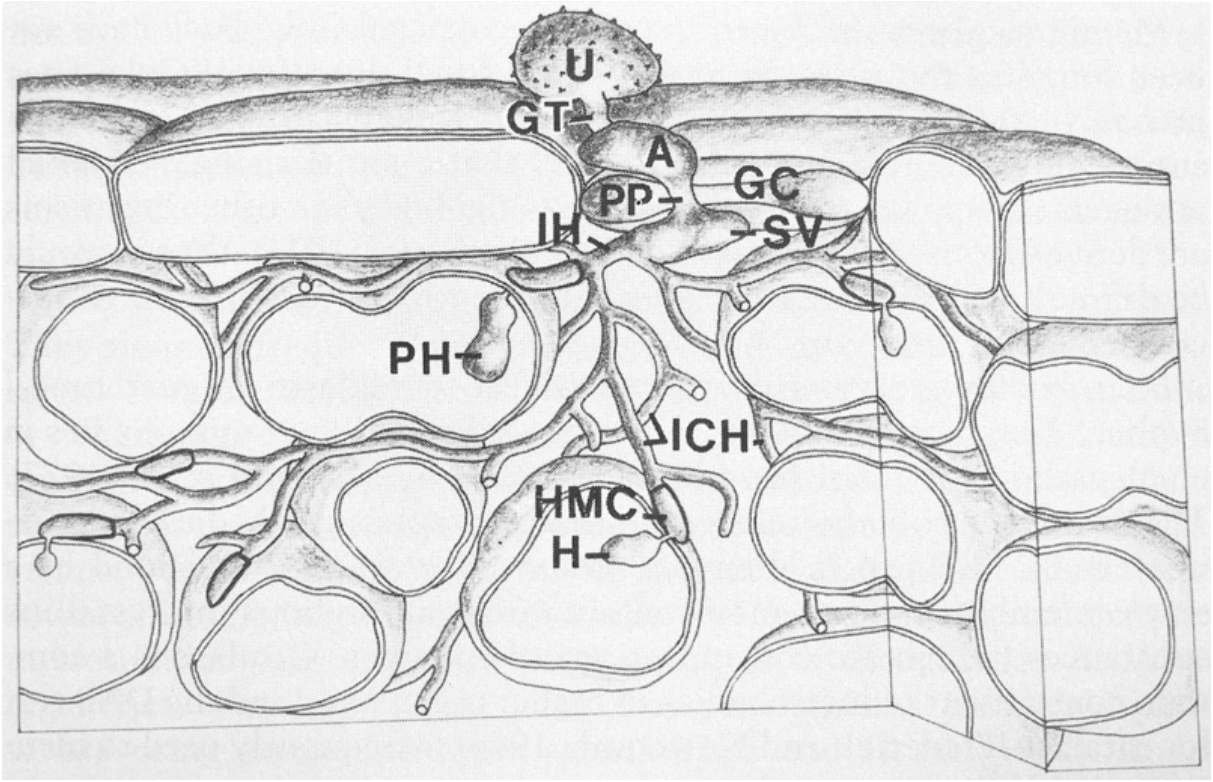


Figure 2. A young rust fungal colony, about 60 hours old, in a cereal leaf. The sequence of development is germination of the urediospore (U), formation of an appressorium (A) from the germ tube (GT) over a stoma, penetration past the guard cells (GC) via a penetration peg (PP), formation of a substomatal vesicle (SV), growth of an infection hypha (IH), formation of the primary haustorium (PH), then branching and growth of intercellular hyphae (ICH), and formation of additional haustoria (H). The haustorium mother cells (HMC) are indicated in bold outline. (Drawn by Dr. J. Chong; Reprinted from Harder 1984).

Boundaries of basal resistance

Basal resistance is a term commonly used by scientific authors to refer to the early defence response of plants to a pathogen attack. Basal defence is the complement of the term of basic compatibility, and refers to a defence system that is not based on a hypersensitive response (Heath 1991). Basic compatibility is the state that results from the capacity of the microbe to deal effectively with the defence that plant species perform against unadapted microbial intruders. The definition of basal resistance is particularly vague in plant pathology and it would be necessary to delimit its physiological boundaries to obtain a uniform discussion within the scientific community. A clear definition of basal resistance is difficult to give

because of our limited knowledge in the actual biological events that happen in the early steps of plant defence. Also, as our understanding advances, the idea that plant defence systems rely on a continuum of layered defences seems to defy man-made boundaries and definitions (Heath 2001; da Cunha et al. 2006).

A non-host or host resistance? Non-host resistance is the resistance shown by an entire plant species to a specific parasite or pathogen (Heath 2000). Non-host resistance to maladapted pathogens can be the result of effective non-specific defences such as physical and constitutive chemical features. For rust fungi, the topography of the leaf surface is an example of physical feature to which the fungus should be adapted for successful colonisation (Vaz Patto and Niks 2001). As for chemical factors, preformed peptides and secondary metabolites are potential deterrents against microbial infection. Such physical and chemical responses are often referred to as the preformed barriers of a non-host plant to its potential pathogens. Additionally, there is ample genetic evidence that non-host resistance also involves inducible defences elicited by the perception of parasite-specific molecules (Heath 2000, 2001; Thordal-Christensen 2003). Jafary et al. (2006a, 2006b) used the barley − *Puccinia* rust model system to show that non-host resistance of barley to various heterologous rust species was under complex genetic control of many genes, each with a considerable level of rust species specificity. Their work also confirmed that non-host resistance and quantitative host resistance in barley to, respectively, heterologous and homologous rust species were mainly based on a pre-haustorial, non-hypersensitive resistance mechanism and that loci for non-host resistance tended to map to loci known to carry also QTLs for host resistance. The difference between non-host and host resistances may reside in the solidity of the recognition leading to resistance (Thordal-Christensen 2003). We propose that the basal resistance of a plant represents the inducible component of non-host resistance that occurs when a pathogen has been able to negate the preformed barriers and has become subject to perception by the plant. This perception is usually triggered via interaction between a plant receptor and a microbe-associated molecular pattern (MAMP), also called pathogen-associated molecular pattern (PAMP). Then, the suppression of basal defence will determine host-cell accessibility to the fungal pathogen (Heath 1991; Panstruga 2003; Caldo et al. 2006). Pathogen molecules may completely fail to suppress plant basal defences, generally resulting in complete basal resistance as in the barley – rye leaf rust (non-host) interaction (Atienza et al. 2004; Niks 1989), or may partially succeed, resulting in a residual basal resistance as in the barley – *P. triticina* (near non-host) or 'Vada' – *P. hordei* (host) interactions (Atienza et al. 2004; Jafary et al. 2006a). Therefore, basal resistance could comprise both non-host and host types of resistance.

A pre- or post-haustorial resistance? For rust fungi, the entry into a host cell to form a haustorium and establish basic compatibility inevitably requires penetration of the plant cell wall. The resistance that prevents the formation of fungal haustoria has been termed pre-

haustorial actions of resistance (Heath 1974), or pre-haustorial resistance, in contrast to the post-haustorial resistance that is usually accompanied by programmed cell death. Then, pre-haustorial resistance is associated with the resistance of the plant to the penetration of the cell wall by the pathogen. This type of resistance results in a polarisation of the host cell towards the site of penetration attempt that typically leads to the formation of cell wall reinforcements, also called cell wall appositions or papillae (O'Connell and Panstruga 2006). The presence of papillae opposite haustorial mother cells probably determines the fate of an infection structure. A correlation between number of papillae and level of aborted infection structures has indeed been reported for the barley – *P. hordei* and for the wheat – *P. triticina* interactions (Niks 1986; Jacobs 1989a, 1989b). It should however be mentioned, that although the major component of papillae is callose, other substances of which they are impregnated, such as polysaccharides, phenolic compounds, reactive oxygen intermediates and proteins, may contribute to the inhibition of fungal growth (Zeyen et al. 2002). Several genes influencing pre-haustorial resistance have been identified, and may inherit qualitatively or quantitatively, depending on the magnitude of their effect (Collins et al. 2007). It seems likely that active signalling between the penetrating rust fungus and the host plant cell will determine the outcome of the attack, i.e. the fungus will form a haustorium into the cell or will fail to do so (Heath 1997). We propose that the basal resistance of a plant is strongly associated with its capacity to prevent cell wall penetration and haustorium formation by the attacking fungus. Nevertheless, Jacobs (1989a) described a post-haustorial growth retardation of *P. triticina* on wheat that was associated neither with formation of papillae nor with hypersensitive cell collapse. Two possible explanations for this retardation of the growth of the rust hyphae are extra-cellular components or the impediment of nutrient uptake through the extra-haustorial matrix into the haustorium. Such defence mechanisms could also be regarded as part of the basal resistance of a plant if based on perception of the pathogen.

New insights into plant innate immunity

The ability to discriminate between self and non-self is a key feature of all living organisms, and it is the basis for the activation of innate immune responses upon microbial infection. The idea that concepts of immunity in animals may have parallels in plants was first formally considered about 75 years ago by Chester (1933). Nearly 50 years later, Clarke and Knox (1979) recognised that in plant species, like in animals, pathogenicity is the exception rather than the rule, and proposed the existence of recognition and defence mechanisms in plants. They tried to get insight into the molecular logic of these mechanisms at a time when "*some progress was being made by a few brave and persistant individuals* (referring to Smith 1978)", and hypothesised the existence of plant receptors for microbial molecules that are located at the cell surface, associated with the cell wall or with the underlying plasma

membrane. Such assumptions, made several decades ago, are only being verified in recent years as much progress is being made on the understanding of the molecular organisation of plant innate immunity (Nürnberger et al. 2004; Chisholm et al. 2006). Da Cunha et al. (2006) described plant innate immunity as a continuum of layered defences raised against pathogen attempts of infection. Depending on their life cycle, the pathogens will have to face different layers of defence. As many other biotrophic pathogens, *P. hordei* must penetrate host cell walls to elaborate haustoria. Penetration of the host cell wall represents the first key step towards the establishment of a compatible interaction between the pathogen and its host (O'Connell and Panstruga 2006). For the host plant, prerequisite to the induction of defence is perception of the pathogenic threat. This perception can be either direct via interaction between a plant receptor and a microbe-associated molecular pattern (MAMP), or indirect via detection by a plant receptor of microbe-induced molecular patterns (MIMP), which are modifications of host-derived molecules orchestrated by the pathogen (Mackey and McFall 2006).

MAMP detection serves as an early warning system for the presence of non-self molecules. A MAMP is a structural element from within any molecule of a potential pathogen that is not present in the host, therefore typically 'non-self', and that is perceived via direct interaction with a host defence receptor (Mackey and McFall 2006). MAMPs can be found in highly conserved molecules, also called general elicitors, that are essential for the viability of the pathogen (Ingle et al. 2006). Nonetheless, plasticity does exist within MAMPs because a pathogen molecule recognised via a MAMP contained within it may evolve in a way that permits it to maintain its function while avoiding interaction with its cognate MAMP-regulator (Andersen-Nissen et al. 2005). Few molecule-containing MAMPs involved in the early detection of pathogens by a plant have been identified (reviewed in Nürnberger and Lipka 2005; Ingle et al. 2006). The best-studied of them is *flg22* that interacts with the extracellular LRR domain of the transmembrane receptor-kinase FLS2 (Gómez-Gómez and Boller 2000). *Arabidopsis* possesses 216 receptor-like kinases (RLK) with an extracellular LRR domain (Shiu and Bleecker 2001), a significant number of which can be expected to be involved in MAMP perception. Interestingly, Shiu et al. (2004) found that rice has nearly twice as many RLKs as *Arabidopsis* does, and that most of this expansion involves resistance-related genes. Nevertheless, if LRR-RLKs are likely to constitute a large proportion of disease receptors that act in response to attempted penetration of the plant cell wall (Gómez-Gómez 2004; Chisholm et al. 2006), they also regulate a variety of developmental and other defence-related processes including cell proliferation, stem cell maintenance, hormone perception, wounding response and symbiosis (Torii 2004). There can be some specificity in the plant response to general elicitors, but not of the order that is expected in view of the apparent plant species or even intra-species specificity of non-hypersensitive resistance described by Jafary

et al. (2006a) for the barley – rusts model system. The explanation might reside with the secretion of specific elicitors by the pathogen, the effector molecules.

Initial detection of pathogen general elicitors through MAMPs and activation of a basal defence response by the plant is only the first step in the plant-pathogen arms race. Plant pathogens also secrete a suite of effectors that collectively promote their virulence in host plants. Effectors manipulate host cell structure and function to actively suppress its defence system but effectors can also be recognised by the plant and elicit a subsequent defence layer. The level of specificity at which effectors suppress the host basal defences shows great variation and might be determined by the host defence determinant targeted by the pathogen. A tempting hypothesis would be that plant defence factors and pathogen effectors have build-up in an evolutionary arms race resulting in an increasing level of recognition specificity. Two classes of effectors target each either the apoplast or the cytoplasm (Kamoun 2006). The primary task of apoplastic effector proteins is related to suppressing host defences localised in the plant extracellular space. If successful in penetrating the plant cell wall, the formation of a functional interface between haustoria and plant cell membrane will permit the pathogen to take up nutrients and to export cytoplasmic effectors inside the plant cell, where they target different subcellular compartments (Mendgen et al. 2000). In oomycetes, these cytoplasmic effector proteins contain a conserved N terminal motif (RxLR) that is essential to cross the host membrane during export from the pathogen haustorium into the plant cell (Tyler et al. 2007). The RxLR motif was however not identified in flax rust Avr proteins (Catanzariti et al. 2006). Some effectors elicit defences because they contain a MAMP. Other effectors elicit defences because they produce a MIMP. A MIMP is the product of the intrinsic activity of an effector that will be recognised through the alteration of the functional state of a host molecule (Mackey and McFall 2006). This can be illustrated by the guard hypothesis, which proposes that the interaction between an R-protein and its cognate Avr determinant is mediated by a host protein that is the target for the effector function of the Avr determinant (Jones and Takemoto 2004). MAMP or MIMP recognition by the host plant receptors can occur in the apoplast before cell wall penetration, or inside the host cell after cell wall penetration. The major class of receptor acting inside the host cell is the well-known NBS-LRR gene family that lead, upon recognition, to programmed cell death.

An intriguing and difficult to resolve question is the estimation of the number of secreted effectors that might be encoded by the pathogen. The genome sequences of several *Phytophthora* species allowed a first glimpse into it and revealed that *Phytophthora* species can encode more than 100 potentially secreted proteins carrying the RxLR motif (O'Connell and Panstruga 2006; Kamoun 2006). In flax rust, 21 genes encoding secreted proteins have been identified in a haustorium-specific cDNA library (Catanzariti et al. 2006). Those effectors are frequently variable between related microbes, even within the same species. Mackey and McFall (2006) compared the collection of effectors produced by a pathogen as a

'tool kit', in which some effectors might be redundant with other effectors or may contribute to virulence on different host species.

What does quantitative resistance mean?

Plant quantitative traits are typically controlled by several genes whose individual effects are small and contribute more or less to the overall level of the measured trait. Although in principle, a quantitative trait could be controlled by only one gene. The study of the inheritance of quantitative traits has become possible with the development of molecular marker linkage maps that allowed to map QTLs to particular genomic regions. A QTL is characterised by its quantitative effect, the size of which depends on the genetic background and on its allelic form. Detection of such a quantitative gene requires QTL-mapping software to establish its position (Niks et al. 2004). A QTL represents a genomic region defined by a confidence interval and can be the result of the effect of a single gene (Tian et al. 2006) or of a complex of genes closely linked with each other (Gao et al. 2004). Each of these genes strictly follow Mendelian principles of inheritance and can be isolated by map-based cloning provided that their effect can be reliably assessed. Indeed, accurate phenotyping remains the key to the successful map-based cloning of QTL-genes.

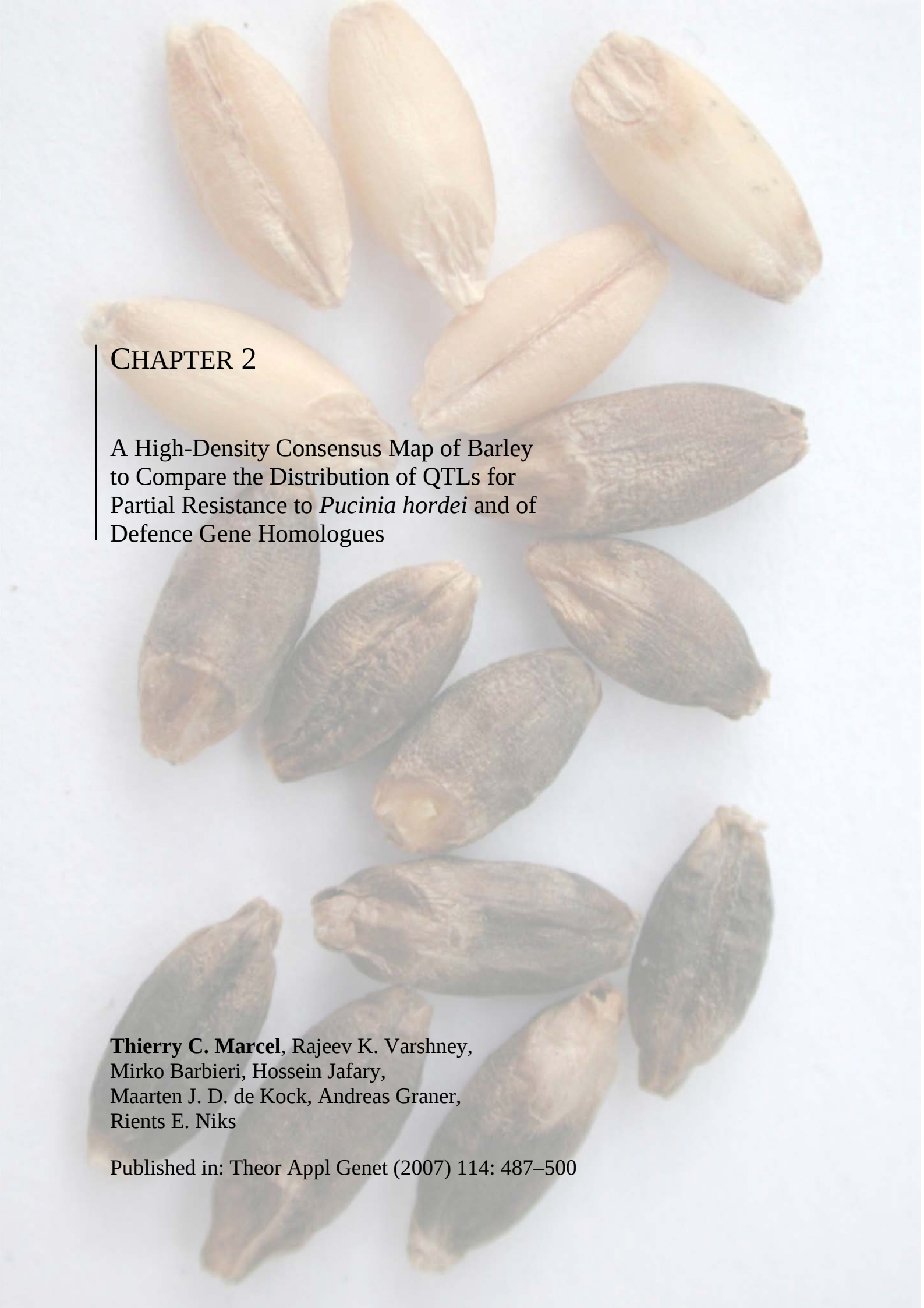
Many terms and concepts have been associated with quantitative resistance, such as slow rusting, field, intermediate, quantitative, incomplete, general, partial, horizontal, adult plant, multigenic, race-non-specific resistance, etc. Quantitative resistance should refer to all types of resistance that behave in a quantitative way, as described in the previous paragraph. Several studies have reported a linkage association between genes for qualitative resistance and loci for quantitative resistance (Geffroy et al. 2000; Bai et al. 2003). Li et al. (1999) reported evidence that a defeated rice resistance gene for hypersensitivity was acting as a QTL against a virulent strain of *Xanthomonas oryzae*. On a genome wide study of rice, Wisser et al. (2005) found also that *R*-genes and *R*-gene analogues (RGA) of the NBS-LRR class were significantly associated with QTLs for multiple diseases. Such evidence strongly suggests that a proportion of the quantitative resistance identified in several plant-pathogen systems results from the action of partially defeated *R*-genes for hypersensitivity. Nevertheless, Wisser et al. (2005) also reported that for a number of QTL regions there was no co-localising major gene or RGA identified, supposing that another proportion of the quantitative resistance cannot be explained by partially defeated *R*-genes.

Parlevliet (1978) described partial resistance of barley to leaf rust as a type of resistance that retards epidemic development in the field, although plants show a high, compatible, infection type. Niks (1982, 1983a, 1986) demonstrated later that this resistance was not based on hypersensitivity and that *P. hordei* was hampered in its ability to penetrate cells and to form haustoria. He considered this as a reduced level of basic compatibility (Niks,

1982), and found similarity with the much stronger non-host resistance (Niks 1983a, 1983b). Qi et al. (1998b) mapped QTLs for partial resistance to barley leaf rust on seedlings and on adult plants of a recombinant inbred line population and compared their position with the ones of known *R*-genes. They did not find any indication that map positions are shared between *R*-genes and QTLs for partial resistance. In that case, partial resistance could be defined as that proportion of quantitative resistance that contributes to the basal defence of the plant against the intruding pathogen. The nature of the genes explaining QTLs for partial resistance remains unknown. Many speculations have been made based on the identification of candidate genes through co-localisation on a linkage map (Faris et al. 1999; Trognitz et al. 2002; Ramalingam et al. 2003; Liu et al. 2004; Wisser et al. 2006) or through differential expression between susceptible and resistant plants (Neu et al. 2003; Gjetting et al. 2004; Zierold et al. 2005). A number of candidate genes have been identified from a multitude of gene families: protein kinases (receptors), WRKY, MYB (transcription factors), peroxidases, chitinases (pathogenesis-related proteins), glutathione *S*-transferase, UDP-glucosyltransferase (role in detoxificaton), etc. But to validate (some of) the candidate genes identified, the actual isolation, cloning of genes underlying QTLs for partial resistance is required.

Objectives of this thesis

The aim of the research presented in this thesis was to develop genetic tools that permit to study the architecture of partial resistance of barley to leaf rust at the genome level and at the gene level. The construction of high-density consensus maps of barley offer a comparative tool between different barley genetic maps in which genes and QTLs have already been placed and provides a wealth of genetic markers that can be used to dissect regions of interest. The development of sets of isogenic lines containing individual or combined QTLs for partial resistance to leaf rust allows the evaluation of QTL effects in a uniform genetic background, partly overcoming the difficulties of identifying QTL phenotypes. Isogenic lines are also indispensable to the construction of segregating populations that are required for substitution mapping and further map-based cloning experiments. We hope that this work will facilitate the map-based isolation of a plant gene for partial resistance.



CHAPTER 2

A High-Density Consensus Map of Barley to Compare the Distribution of QTLs for Partial Resistance to *Pucinia hordei* and of Defence Gene Homologues

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Variation of seeds colour in the L94 x ‘Vada’ population

A high-density consensus map of barley to compare the distribution of QTLs for partial resistance to *Puccinia hordei* and of defence gene homologues

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Abstract A consensus map of barley was constructed based on three reference doubled haploid (DH) populations and three recombinant inbred line (RIL) populations. Several sets of microsatellites were used as bridge markers in the integration of those populations previously genotyped with RFLP or with AFLP markers. Another set of 61 genic microsatellites was mapped for the first time using a newly developed fluorescent labelling strategy, referred to as A/T labelling. The final map contains 3,258 markers spanning 1,081 centiMorgans (cM) with an average distance between two adjacent loci of 0.33 cM. This is the highest density of markers reported for a barley genetic map to date. The consensus map was divided into 210 BINs of about 5 cM each in which were placed 19 quantitative trait loci (QTL) contributing to the partial resistance to barley leaf rust (*Puccinia hordei* Otth) in five of the integrated populations. Each parental barley combination segregated for different sets of QTLs, with only few QTLs shared by any pair of cultivars. Defence gene homologues (DGH) were identified by tBlastx homology to known genes involved in the defence of plants against microbial pathogens. Sixty-three DGHs were located into the 210 BINs in order to identify candidate genes responsible for the QTL effects. Eight BINs were cooccupied by a QTL and DGH(s). The positional candidates identified are receptor-like kinase, *WIR1* homologues and several defence response genes like peroxidases, superoxide dismutase and thaumatin.

Additionnal keywords: *Hordeum vulgare*; leaf rust; candidate gene analysis; simple sequence repeat (SSR); gene-targeted markers (GTM); RECORD; skewed segregation

Introduction

Linkage maps are essential tools in identifying genes responsible for polymorphic traits like disease resistance versus susceptibility, for comparing the genomes of different species, for map-based gene isolation and for genome sequencing. The earliest type of molecular markers used to construct genetic linkage maps were restriction fragment length polymorphisms (RFLP), which were applied in barley to genotype the Igri × Franka and the Steptoe × Morex populations (Graner et al. 1991; Kleinhofs et al. 1993). Nowadays, RFLPs have largely been replaced by different types of PCR-based molecular markers such as amplified fragment length polymorphisms (AFLP), single nucleotide polymorphisms (SNP) and single sequence repeats (SSR). The AFLP technology was used in barley to genotype the Oregon Wolfe Barley, L94 × Vada, SusPtrit × Vada and SusPtrit × Cebada Capa populations (Costa et al. 2001; Qi et al. 1998a; Jafary et al. 2006a, 2006b). The so-called expressed sequence tags (EST) are one of the most informative sources of genetic markers because they represent partial sequences of genes and hence, those markers should map at the position of the corresponding gene. The RFLP, SNP and SSR technologies are actually used to saturate the barley genome with EST-based markers (Thiel et al. 2003: SSR; Sato et al. 2004: SNP; Rostoks et al. 2005: SNP, SSR; Varshney et al. 2006a: SSR; Stein et al. 2007: RFLP, SNP, SSR). Then, one of the greatest challenges is the integration of these different maps, genotyped by several groups using different techniques and different mapping populations, to produce a unified picture of the barley genome. In the past, two consensus maps based on RFLP markers (Langridge et al. 1995; Qi et al. 1996), containing 587 and 880 markers, respectively, and one consensus map combining 700 RFLP, AFLP and SSR markers (Karakousis et al. 2003) were constructed for barley.

The most important use for linkage maps is to identify chromosomal locations containing genes and quantitative trait loci (QTL) associated with traits of interest. QTL analysis provides a means to map several loci and to determine their interactions in a segregating cross (Borevitz and Chory 2004). Understanding the response of QTLs in different environments or genetic backgrounds can lead to the development of improved crop varieties through marker-assisted selection. If the genes underlying the QTL are known (i.e. the QTL has been “cloned”), then transgenic approaches can also be used to directly introduce beneficial alleles across intra- or inter-species boundaries (Borevitz 2004). Nevertheless, although map-based positional cloning has been used to isolate a large number of genes that inherit according to Mendelian ratios, such cloning is considered problematic for QTLs since genotypes cannot be unambiguously recognised from phenotypes of individual plants (Remington et al. 2001). Notably, this is the case for QTLs involved in disease resistance. An alternative approach to positional cloning of those QTLs is the candidate gene approach. The most common way to identify a candidate gene that may affect the QTL for resistance directly

is to look for map cosegregation between genes of interest and QTLs for resistance (Pflieger et al. 2001). This approach has been applied in several experiments for different plant–pathogen systems (Faris et al. 1999; Wang et al. 2001; Trognitz et al. 2002; Ramalingam et al. 2003; Lanaud et al. 2004; Liu et al. 2004). However, in the end, it always remains to be determined whether the candidate gene and the QTL map on the same position on the linkage map by chance or indeed because the candidate gene really is responsible for the phenotype determined by the QTL. The process of identifying candidate genes relies on the available information gained through the mapping of QTLs and of gene sequences with known function. Since biological functions are attributed to an increasing number of gene sequences, keeping gene annotations up to date with current publications is an important task.

In this paper, we report the merge of the available linkage mapping data of six different barley populations with mapped QTLs for partial resistance to barley leaf rust (*Puccinia hordei* Otth) and defence gene homologues.

Materials and methods

Plant material (mapping populations)

Three recombinant inbred line (RIL) populations and three doubled haploid (DH) populations were used to construct a consensus map of barley. The RIL populations have been developed at Wageningen University (Wageningen, The Netherlands), and consist of lines derived from crosses between L94 and Vada (L × V, 103 lines; Qi et al. 1998a), between SusPtrit and Vada (Su × V, 152 lines; Jafary et al. 2006a), and between SusPtrit and Cebada Capa (Su × CC, 113 lines; Jafary et al. 2006b). The two DH populations consisting of lines derived from crosses between Steptoe and Morex (St × M, 150 lines; Kleinhofs et al. 1993) and between *Dom* and *Rec* (OWBs, 94 lines; Costa et al. 2001), have been developed in North America and are reference mapping populations subject to extensive genotyping and phenotyping. The third DH population consists of lines derived from a cross between Igri and Franka (I × F, 71 lines; Graner et al. 1991), which were used to construct the first complete RFLP linkage map of barley.

Available linkage mapping data

The available data sets of the three RIL populations consisted predominantly of AFLP markers (Table 1). For L × V, the segregation data of 568 markers were obtained from Qi et al. (1998a). For Su × V and Su × CC, the segregation data of 450 markers and of 506 markers, respectively, were obtained from Jafary et al. (2006a, 2006b). The segregation data sets of the OWBs were downloaded from the Oregon State University (OSU) Barley Project web site

(http://www.barleyworld.org/). Most of the 769 markers downloaded for the OWB population are AFLP markers (Table 1). The segregation data sets of the St × M and I × F populations were downloaded from the publicly available GrainGenes 2.0 databank (http://wheat.pw.usda.gov/GG2/index.shtml). Those two data sets comprised 588 and 550 markers, respectively, and consisted predominantly of RFLP markers (Table 1).

Table 1. Characteristics of the six barley populations used to construct the consensus map and numbers of marker loci and defence gene homologues (DGH) placed on the consensus map per molecular marker type

Barley populations			Number of markers within populations							DGH no.[3]
Name	Type	Lines	RAPD	RFLP	AFLP	SSR	Gene[1]	Other[2]	Total	
L×V	RIL	103	0	0	785	138	5	29	957	11
Su×V	RIL	152	0	0	420	24	2	4	450	4
Su×CC	RIL	113	0	0	481	14	0	0	495	0
OWBs	DH	94	5	103	594	76	14	5	797	23
St×M	DH	150	11	421	0	177	11	15	635	21
I×F	DH	71	0	476	0	74	7	0	557	8

[1] The class "gene" comprises isozyme and morphological markers, and major disease resistance genes

[2] The class "other" comprises simple PCR markers such as SNP, SCAR and CAPS

[3] Number of defence gene homologues mapped in each population

Genetic mapping of PCR markers

DNA extraction was done according to the CTAB-based protocol of Steward and Via (1993), adjusted for 96-well format.

We scored an additional 235 AFLP markers segregating in L × V with 11 *Pst*I/*Mse*I primer combinations. The AFLP procedure was essentially performed as described by Vos et al. (1995) with some modifications according to Qi and Lindhout (1997). The selective *Pst* primer was labelled with IRD700 or IRD800 and the AFLP fingerprints generated on a LICOR 4200 DNA sequencer (LI-COR® Biosciences, Lincoln, NE, USA). The following primer combinations were run: P14M50, P14M54, P14M56, P14M61, P15M47, P15M51, P15M52, P15M53, P16M50, P16M51 and P17M47, following the nomenclature proposed by Qi and Lindhout (1997) and Bai et al. (2003). Additional primers were M52 (M00 + CCC), M53 (M00 + CCG), M56 (M00 + CGC), P16 (P00 + CC) and P17 (P00 + CG).

A set of simple PCR markers was developed and used to genotype the L × V or Su × V population. This set consists of 6 sequence characterised amplified region (SCAR) markers, 26 cleaved amplified polymorphic sequence (CAPS) markers and 1 derived CAPS (dCAPS: Neff et al. 1998). Primers for 16 of those SCAR and CAPS markers were developed based on DNA sequences of barley genomic clones publicly available in the GrainGenes databank, i.e.

ABG-, BCD-, MWG-, Hor2 and Prx2. Primers for the 17 other SCAR and CAPS markers were developed based on DNA sequences of barley ESTs downloaded from the TIGR Gene Indices database (http://www.tigr.org/tdb/tgi/). Those EST-based markers were named WBE- for Wageningen Barley ESTs. The primer design and polymorphism detection was done as described in Marcel and Niks (2004) using the Lasergene software package (DNASTAR Inc., Madison, WI, USA). Detailed information on the 33 SCAR and CAPS markers presented in this study is available online as Table ESM S1.

We used SSR markers to integrate the maps of the six barley populations. The L × V and the St × M populations were genotyped with 89 and 21 polymorphic SSR markers, respectively. The segregation data for 20 additional SSRs genotyped in St × M and 11 SSRs genotyped in OWB were obtained from Varshney et al. (2006a). The HV-, Bmac-, Bmag-, EBmac- and EBmag- markers were amplified according to the PCR protocols reported by Ramsay et al. (2000) and the GBMS- and GBM- markers according to the protocol described by Thiel et al. (2003). The primers were synthesised and the reverse primers IRDye-labelled at Biolegio BV (Nijmegen, The Netherlands). The PCR product was visualised on LICOR 4200 DNA sequencer. Additionally, a polymorphism test with 313 unmapped SSR primer combinations (GBM-) developed at IPK (Gatersleben, Germany) revealed 74 polymorphic markers in L × V and/or in Su × V (i.e. 24%). From these 74 SSRs, 13 pairs of markers were associated with the same consensus sequence resulting in a set of 61 unigene-based markers. Subsequently, we mapped 49 of those GBM markers in L × V and 12 in Su × V. The primer combinations of those 61 SSRs were unlabelled. Therefore, their PCR amplification products were fluorescently labelled according to the A/T labelling procedure before loading on gel.

A/T labelling procedure

DNA polymerases without proofreading activity generally catalyse the addition of a 3'-terminal deoxyadenosine to a PCR amplification product (Clark 1988). This 3' overhang of an adenosine residue in a PCR amplification product is widely used for universal cloning into a vector with a 3'-thymidine overhang (Magnuson et al. 1996; Zhou et al. 1995; Promega). Here, we used this strategy to fluorescently label PCR amplification products produced by SSR primer combinations with an adapter containing the appropriate IRDye to allow infrared detection during electrophoresis. The PCR amplification product of an SSR primer combination (5 µl) was ligated O/N at 37°C to the IRDye-labelled T-adapter in a ligation mixture containing 1 Unit *T4 Ligase* (Invitrogen), 1 pmol IRDye-700 labelled T-adapter, 2 nmol ATP, 0.25 Units *Supertaq* and 1.5 µl 5× T4-ligation buffer (Invitrogen) in a total volume of 10 µl. The T-adapter is generated by mixing equal amounts of the oligo's adT-top [$_{700}$GACTGCGTACCAATTCACT, near-infrared fluorescently labelled, (Biolegio, The Netherlands)] and adT-bot ($_{P}$GTGAATTGGTACGCAGT^{NH2}). The bottom strand (adTbot)

contains a 5'-terminal phosphate group for efficient ligation and a 3'-terminal amine group to avoid A-tailing of the adapter.

Construction of the barley consensus map

The quality of the data sets was estimated by running a Chi-square test for the segregation data of each marker. Then, we ordered twice markers within individual data sets with the program RECORD (Isidore et al. 2003). After each marker ordering by RECORD, conflicting data points (i.e. singletons) and other potential errors in the marker segregation data were identified and replaced by missing values as suggested by Isidore et al. (2003). A new improved version of JoinMap (JoinMap 4) based on a faster algorithm (Jansen et al. 2001), kindly provided by Dr. Van Ooijen (http://www.kyazma.nl/), was subsequently used to calculate the six individual barley maps. Then, the integrative function of the software package JoinMap® 3.0 (Van Ooijen and Voorrips 2001) was used to construct a framework map containing only the bridge markers identified between two or more populations. The map distances were calculated using the Kosambi mapping function. Next, the six individual barley maps were recalculated by adding the order of the framework markers, as given by JoinMap® 3.0, as a "fixed order file" into JoinMap 4. The final consensus map was calculated by using the framework map as fixed backbone onto which the unique loci of each individual map were added following the "neighbors" map approach described by Cone et al. (2002). The obtained consensus map was divided into 210 BINs of about 5 cM each. For the sake of continuity of the system, we maintained as much as possible the BIN-defining markers of Kleinhofs and Graner (2001) in their role in the present map and they kept the same BIN number (e.g. 1H_01 for chromosome 1H BIN number 01). The BINs in the latter map span about 10 cM. Each 10 cM BIN was then subdivided into two 5 cM BINs in order to obtain a greater precision allowed by the high marker density of our map (e.g. 1H_01.1 and 1H_01.2).

Nomenclature of the markers

The AFLP marker loci were assigned with a primer combination code followed by the fragment size as described by Qi and Lindhout (1997) and Bai et al. (2003). The nomenclature of the SSR markers was described in detail in Varshney et al. (2007). The GBR-, GBS- and GBM- markers had been developed at IPK (Gatersleben, Germany) and correspond to Gatersleben Barley RFLP, SNP and microsatellite markers, respectively. The prefixes, "i", "m" and "d" were added to marker names to indicate isozyme markers, morphological markers and major disease resistance genes following a Mendelian segregation, respectively. Multiple segregating bands identified with a single probe or one primer combination were indicated with higher case letters for RFLP markers (e.g. ABC151A and ABC151B) and with

lower case letters for SSR markers (e.g. Bmac0040a and Bmac0040b). The rest of the marker names remained unchanged compared to their record in GrainGenes 2.0.

Mapping strategies of defence gene homologues (DGHs)

In the present paper we use the term “resistance gene” for genes that specifically confer a vertical resistance in race-cultivar-specific interactions, like *Rph*- genes to *P. hordei*. A “defence gene” is more generally induced in a plant response to a pathogen challenge, such as pathogenesis-related (PR) proteins. Analogues of resistance (RGA) and defence genes (DGA) are genes isolated using a PCR approach with degenerate primers designed from conserved domains of plant resistance and defence genes, respectively (Lanaud et al. 2004). Homologues of resistance genes (RGH) are genes identified by blast analysis that shares significant identity of the amino-acid sequence with known resistance genes (Monosi et al. 2004). Following the same nomenclature, we named the genetic markers derived from ESTs homologous to known defence genes Defence Gene Homologues (DGH).

A list of genes involved in the partial and/or basal resistance of plants to fungal pathogens was drawn up based on the information available in the scientific literature. We also considered the defence genes as well as a few resistance genes which were differentially expressed between susceptible and partial or non-host resistant barley lines (Neu et al. 2003; Gjetting et al. 2004; Zierold et al. 2005; Jafary et al. unpublished data). We selected a total of 81 defence genes and five resistance genes that might explain the QTLs for partial resistance to barley leaf rust reported in this study (Table ESM S2). The selected defence and resistance genes were tBlastx in the TIGR Gene Indices database. A barley EST was considered homologous to the gene used for tBlastx when annotated with a similar function in TIGR database and at a treshold *E* value $\leq 10^{-5}$. The blast analysis resulted in the identification of 245 homologous barley unigenes. For convenience, all the homologues of the 81 defence and five resistance genes selected are considered as DGH in this paper. Three strategies were followed to map a maximum of DGHs on the developed barley consensus map. The first strategy consisted in developing simple PCR markers based on the unigene sequences obtained by blast analysis in TIGR. In the second strategy, we searched the transcript map of barley, which is being developed at IPK, for mapped DGH sequences (Stein et al. 2007). The third approach consisted simply in searching the literature for DGHs already placed on one of the maps used to construct the present consensus map.

Disease evaluations at seedling plant stage

The long-time standard barley leaf rust isolate 1.2.1 (*P. hordei* Otth) was used to evaluate the level of partial resistance of the 150 DH lines of St × M and of the 94 DH lines of the OWBs

at seedling stage in a greenhouse compartment. For St × M, the disease experiments were conducted in six replications in time and within each replication one seedling of each DH line was inoculated. For the OWBs, the disease experiments were conducted in three replications in time and within each replication three seedlings of each DH line were inoculated. The seeds were sown in trays of 37 × 39 cm, each of them containing two rows of 10–15 seeds. In each tray one seed of each parental line, Steptoe and Morex or *Dom* and *Rec* and of the control lines, L94 and Vada, were sown. The inoculation was performed as described by Qi et al. (1998b) with about 200 spores per cm^2. The latency period (LP) on each seedling was evaluated and the relative latency period (RLP50S) was calculated, relative to the LP on L94 (Parlevliet 1975).

Statistical analysis

The pedigree analysis of Steptoe and of Morex was realised with the Peditree software package (Van Berloo and Hutten 2005). The wide sense heritability (h^2) for RLP50S was estimated from ANOVA in the St × M and the OWB populations according to the formula $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2 / n)$ where n represents the number of replicates per line. ANOVA on RLP50S revealed significant genotype and replication effects in both populations. Therefore, the genotype effect of each line was extracted from the analysis of variance and its distribution tested for normality. The genotype effect was used to map QTLs on the skeletal maps "St × M basemap" and "OWBbase" (approximately 5–10 cM per marker interval) downloaded from the GrainGenes 2.0 website and from the OSU Barley Project website, respectively. The ANOVA was performed with the GenStat® 8.1 software package (VSN International Ltd. 2005). QTL-mapping was performed using MapQTL® 5.0 (Van Ooijen 2004) according to Qi et al. (1998b). A LOD threshold value of 3.1 was set for declaring a QTL (Van Ooijen 1999) and a two-LOD support interval was taken as a confidence interval for a putative QTL (Van Ooijen 1992). The Restricted-MQM program was run to estimate the proportion of explained phenotypic variance and the effect of the alleles from each parent.

The distribution of QTLs and DGHs on the consensus map was analysed by considering a BIN as "occupied" by a QTL when containing the corresponding peak marker or "occupied" by a DGH(s) when containing the corresponding molecular marker(s). A Chi-square test was realised to test the null hypothesis assuming independent distribution of BINs occupied with a QTL and BINs occupied with a DGH(s).

Results

A high-density consensus map of barley

We used barley SSR markers to link barley populations genotyped with RFLP and barley populations genotyped with AFLP markers. A barley consensus map was constructed, which integrates 3,258 markers. This new consensus map of barley covers a total genetic distance of 1,081 cM with an average distance between two adjacent loci of 0.33 cM. This is the highest density of markers reported for a barley genetic map to date. After the primary inspection of the data, 49 markers were removed because of their skewed segregation. From all singletons detected, 72% were removed after the first marker ordering and the others 28% after the second marker ordering. The data set containing most singletons was the one of OWB with 2.3% of its total number of data points replaced by unknown values, while the data sets of Su × V, Su × CC, L × V, St × M and I × F contained respectively 1.4, 1.2, 0.5, 0.4 and 0.2% of singletons. One gap remained on chromosome 6H of the St × M map only. On the original St × M map (Hordeum-NABGMP1, GrainGenes 2.0) 42.7 cM separate the RFLP markers ABC170A and MWG798A at the telomeric end of 6HL. We tried to map markers within this interval to improve the map integration, but we did not succeed to reduce this gap to less than 30 cM. The framework map contained 50, 95, 89, 53, 70, 66 and 79 integrated bridge markers for the barley chromosomes 1H, 2H, 3H, 4H, 5H, 6H and 7H, respectively. It covered 1,028 cM with an average marker distance between two adjacent loci of 2.08 cM. The correctness of the final consensus map was evaluated by comparing the BIN markers order with the order of the same markers on the BIN map of Kleinhofs and Graner (2001). Marker orders between the maps were in good agreement with solely two inversions of markers on chromosome 3HS and at the distal end of chromosome 5HL. Chromosomes 3HS and 5HL were recalculated by adding the BIN markers of Kleinhofs and Graner (2001) as fixed order in JoinMap® 3.0. The full version of the consensus map is available as an Excel file in Table ESM S3. The chromosomal assignment, the genetic position, the type of marker, the BIN number and the map(s) of origin are given for each marker.

Skewed segregation of molecular markers in six barley populations

Clusters of markers with skewed segregation were identified in all six barley linkage maps used for this study and on all seven barley chromosomes (Fig. 1). The distribution pattern of chromosomal regions associated with skewed marker segregation was different from one map to another. Xu et al. (1997) proposed to regard a chromosomal region as being associated with skewed segregation when four or more closely linked markers are significantly and consistently deviating from the 1:1 ratio. By following this proposition we associated

approximately 75% of the consensus map with regions skewed in one or more population(s). The number of skewed markers varied from 10% of the markers mapped in the OWB population to 41% of the markers mapped in the I × F population. The I × F population also stood out by having the most extreme marker skewness, on chromosome 3H towards the alleles of Igri (allele B) (Fig. 1). In both Su × V and Su × CC map regions of skewed segregation were observed on all the seven barley chromosomes. It is remarkable that the markers were predominantly skewed towards the Vada-allele (allele B) in Su × V while they were predominantly skewed towards the SusPtrit allele (allele A) in Su × CC.

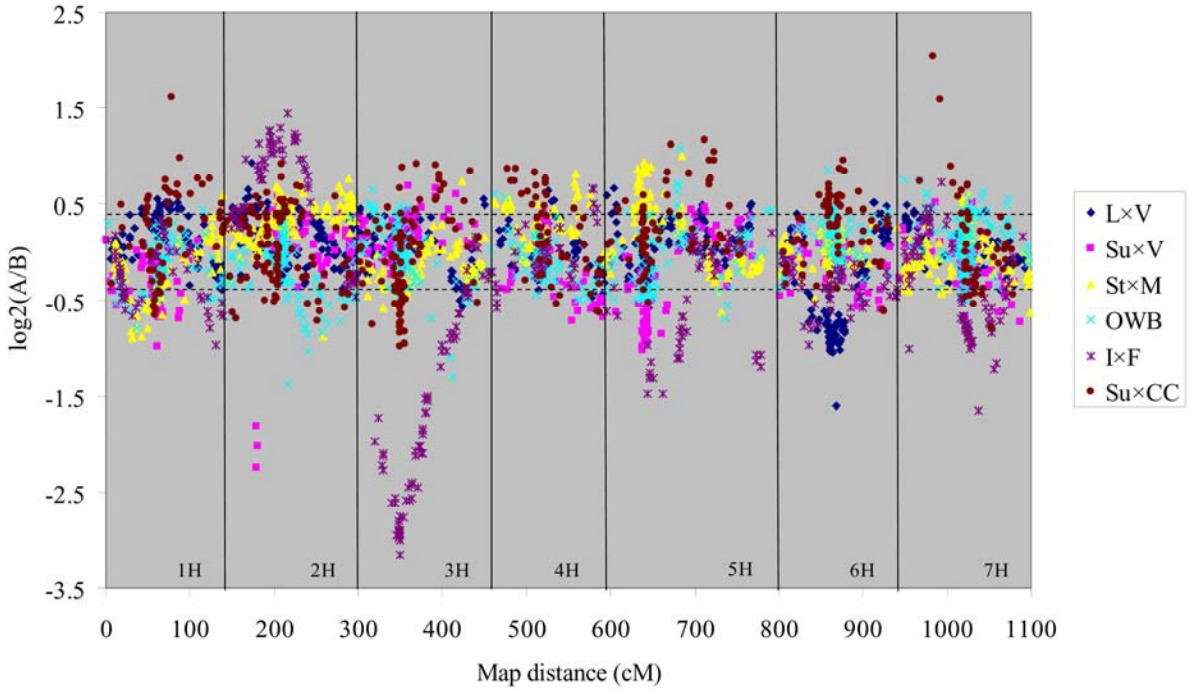


Figure 1. Scatter plot representing the distribution of marker skewness on the six individual barley maps, each *dot* representing one molecular marker. The chromosomes are represented by *solid vertical lines* along the map distance (*x*-axis) and their number is indicated. The log2(*A*/*B*) (*y*-axis) is the log2 value of the ratio of the number of RILs carrying the allele of parental line *A* on the number of RILs carrying the allele of parental line *B*. Markers outside the two *dashed horizontal lines* are significantly skewed as calculated by chi-square test.

Map position and characteristics of gene-targeted markers

A higher level of polymorphism was obtained with genomic SSRs than with genic SSRs. In L × V, 82% of the genomic SSRs tested (HVM-, Bmac-, Bmag-, EBmac-, EBmag- and GBMS-) and 52% of the genic SSRs tested (Hv*GeneName* and GBM-) were polymorphic while in St × M 81% of the genomic SSRs and 37% of the genic SSRs tested were polymorphic. A set of 61 GBM- SSR markers was mapped for the first time in this study. Those 61 markers were

distributed over the 7 chromosomes, which contained each between 5 and 14 of them (Table ESM S4). Since the GBM primers have been developed on barley EST sequences, this new set of SSR markers represent 61 unique genes for which a map position is now available. The PCR-mixtures of the SSR markers analysed were successfully fluorescently labelled, following the A/T labelling procedure.

A list of 81 defence genes and 5 resistance genes that possibly explain the mapped QTLs for partial resistance was drawn up (Table ESM S2) and tBlastx was executed in the TIGR Gene Indices database. For 33 of those genes, 63 barley homologues (Table ESM S5) were mapped in one or more of the barley population(s) used to construct the consensus map. The number of those DGHs per chromosome ranged from 3 for chromosomes 1H and 4H to 18 for chromosome 2H. This suggests a very uneven distribution of the DGHs-based markers over the barley chromosomes. On average, less than one DGH per 45 cM was found on chromosomes 1H, 4H and 5H while one DGH per 8–15 cM was found on the four other chromosomes. Many of the mapped barley DGHs were organised in clusters composed of homologous genes. Those clusters occur for peroxidase like-genes on both arms of chromosome 2H (p*WIR3*, *Per2*, *Prx8* and *Prx2*), for beta-glucanase like-genes on chromosome 3H (*HvNR-R1* and *Glb33*) and for thaumatin like-genes on chromosome 7H (p*WIR232*).

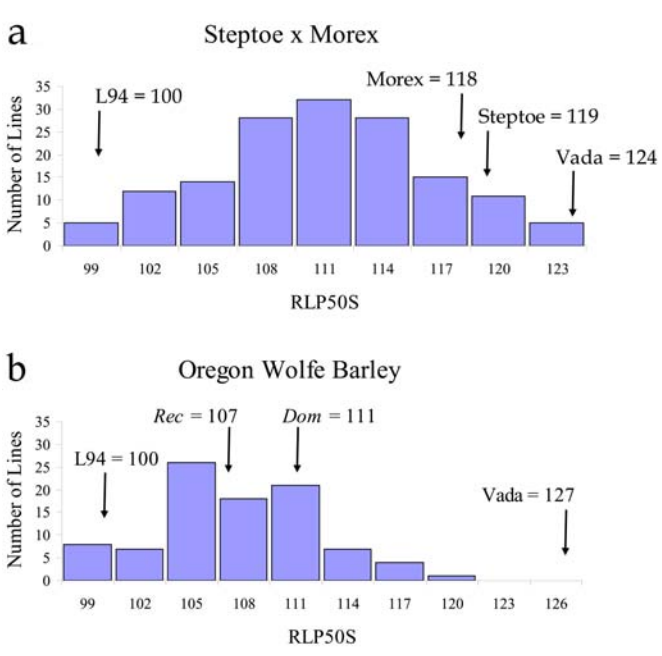


Figure 2. Frequency distribution of phenotypes for the relative latency period of leaf rust isolate 1.2.1 in seedlings (RLP50S) of the Steptoe × Morex population (**a**); and of the Oregon Wolfe Barley population (**b**). Values of the parental and control lines are shown by an *arrow*. The values indicated on the *x*-axis are the midvalues of each class.

QTLs for partial resistance in seedlings of St × M and OWB

The wide sense heritability (h^2) for RLP50S was 0.83 in St × M and 0.84 in the OWBs. On the two populations the RLP50S values covered about the range between the susceptible line L94

and the partially resistant line Vada (Fig. 2). However, in both populations, the RLP50S values for the parental lines were intermediate and similar to each other, indicating transgressive segregation, which implies that both parents contributed alleles for resistance. The genotypic effect used for QTL analysis followed a normal distribution in both populations, as expected in case of polygenic and quantitative resistance.

Table 2. Summary of QTLs conferring partial resistance against leaf rust isolate 1.2.1 at seedling development stage in two barley populations

Steptoe × Morex						Oregon Wolfe Barley					
QTL	Chr.	cM[1]	LOD	Exp%[2]	Add[3]	QTL	Chr.	cM	LOD	Exp%	Add[4]
Rphq8	7H	86.5	3.4	4.1	-1.16	*Rphq12*	2H	124.4	5.7	5.6	-1.11
Rphq11	2H	95.1	21.0	34.1	3.31	*Rphq16*	5H	160.0	13.9	32.7	2.70
Rphq14	1H	11.6	9.6	12.9	-2.00	*Rphq17*	3H	52.2	6.6	10.6	1.55
Rphq15	6H	25.1	5.3	5.5	1.31	*Rphq18*	2H	53.6	5.1	6.9	-1.22
						Rphq19	4H	57.5	4.2	7.6	-1.32
Total				**56.6**	**1.46**	**Total**				**63.4**	**0.60**

[1] Position of the peak marker on the consensus map (in centiMorgan)

[2] Proportion of the explained phenotypic variance

[3] Additive effect of the allele from Steptoe; an effect of 1 is equivalent to a prolongation of the latency period of the rust fungus of 1.72 hour; a negative sign indicates that the resistance allele has been contributed by Morex

[4] Additive effect of the allele from *Dom*; an effect of 1 is equivalent to a prolongation of the latency period of the rust fungus of 1.44 hour; a negative sign indicates that the resistance allele has been contributed by *Rec*

Four QTLs were detected in the St × M population and five in the OWB population (Table 2). Three of the nine detected QTLs were at a mapping position similar to a QTL reported by Qi et al. (1999, 2000) in two other mapping populations. We assume that they are at the same loci and provisionally use the gene designation of Qi et al: *Rphq8* in L × V (Qi et al. 1999) and *Rphq11* and *Rphq12* in L94 × 116-5 (Qi et al. 2000). The six other QTLs were at locations in which no QTL for resistance to *P. hordei* had been reported before. We designated them provisionally as *Rphq14* to *Rphq19*. In St × M, *Rphq11* on chromosome 2H and *Rphq14* on chromosome 1H had the greatest effect on the resistance, while in OWB *Rphq16* on chromosome 5H was the most effective QTL. The other QTLs contributed moderately to the level of partial resistance. Together, the QTLs identified explained 56 and 63% of the phenotypic variation in St × M and OWB, respectively. As expected from the transgressive segregation observed in Figure 2, in both populations the two parents contributed QTLs with resistance alleles and QTLs with susceptibility alleles (Table 2).

Table 3. Chi-square test on the probability of independent distribution of QTLs and DGHs over BINs on the consensus genetic map of barley

Class[a]		Observed results (O)	Expected results (E)		$(O-E)^2 / E$
QTL	DGH				
0	0	154	(189*167)/210	= 150.3	0.091
0	1	35	(189*43)/210	= 38.7	0.353
1	0	13	(21*167)/210	= 16.7	0.819
1	1	8	(21*43)/210	= 4.3	3.183
					$\chi^2 = 4.446$[b]

[a] A class 0 indicates BINs unoccupied by QTL and/or DGH and a class 1 indicates BINs occupied by a QTL and/or by one to several DGH(s)

[b] With a number of degree of freedom (*df*) = 1 the null hypothesis is rejected with a probability $P < 0.05$

Map-based selection of candidate genes to explain the QTLs

In this paper BINs were used to compare the position of 19 QTLs for partial resistance to barley leaf rust with the position of 63 DGHs possibly involved in the defence of plants to fungal pathogens (Fig. 3). Nine of the QTLs were detected in this study on the St × M and OWB populations while the other ten QTLs had been detected previously on L × V (Qi et al. 1998b, 1999), Su × V (Jafary et al. 2006a) and Su × CC (Jafary et al. 2006b; including L94 × 116-5, Qi et al. 2000). An identical name was assigned to QTLs mapped in two or more populations which had overlapping confidence intervals. A BIN containing the peak marker of a QTL was considered as "occupied". Since a QTL mapped in several populations usually had in each population a different peak marker, one QTL could occupy more than one BIN. Similarly, a BIN containing one or more DGH(s) was considered as "occupied". The 19 QTLs occupied 21 BINs and the 63 DGHs occupied 43 BINs. Eight BINs were co-occupied by a QTL and by a DGH(s): *Rphq6* with WBE105 (peroxidase); *Rphq18* with Pox, GBR1062, GBR0126 (peroxidase), GBR0239 (Lipid transfer protein) and GBS0864 (WIR1 protein homologue); *Rphq2* with Prx2, WBE111 and GBR1182 (stress-related peroxidase); *Rphq3* with WBE103, GBS0164 (superoxide dismutase) and with WBE201 (serine/threonine-protein kinase Pelle); *Rphq1* with GBR0202 (PR-1 protein); *Rphq8* with WBE101 (HvNR-F1); *Rphq9* with GBR0192 (LR10 resistance like-protein). *Rphq4* and WBE108 (thaumatin like-protein) were mapped next to each other in the consecutive BINs 5H_02.1 and 5H_02.2, which was considered as a case of co-occupation.

We tested by Chi-square test the null hypothesis assuming an independent distribution of BINs occupied by a QTL and BINs occupied by a DGH(s) (Table 3). The null hypothesis was rejected with a low probability, suggesting a significant tendency to association between QTLs and DGHs position over the consensus map of barley.

Discussion

Properties and usefulness of the high-density consensus map of barley

The final consensus map comprising all 3,258 markers was calculated by combining the use of traditional software packages (Excel, JoinMap® 3.0) with the use of recently developed software packages (RECORD, JoinMap 4). The marker order was always under control of fixed-order files, extracted from the framework map, to guarantee that the integrated marker order remained in agreement with the marker order as observed in the individual maps. The alignment of the individual maps calculated in JoinMap 4 without fixed-order files revealed very few and limited marker reordering between the maps (data not shown). This is an indication that the marker order is very stable, which can only be achieved when the data are almost free of errors. We believe that the visual inspection of the data sets for the identification of errors and replacement of singletons by missing values, as proposed by Isidore et al. (2003), plays a significant role in the stability of the marker order.

AFLP and RFLP markers are the most abundant marker types on this high-density consensus map, respectively 60 and 26% of the total number of markers. The SSR marker system was used to map bridge markers between the populations mainly genotyped with AFLP markers (L × V, Su × V, Su × CC and OWB) and the ones mainly genotyped with RFLP markers (St × M and I × F). SSR markers represent 11% of the total number of markers on the consensus map. Both RFLP and SSR markers are highly transferable between populations of the same species but also between species of the same family. On the other hand, the transferability of AFLP markers is limited to the same plant species. However, even if common AFLP markers can be identified among populations (Qi and Lindhout 1997) and used to align genetic maps (Rouppe van der Voort et al. 1997), the transferability of AFLP markers among laboratories remains disputable. We took as criteria for the selection of potentially common AFLP markers across populations the co-migration of amplification products obtained with identical primer combinations and the localisation of markers to similar map positions. Between the L × V, Su × V and Su × CC linkage maps, developed in the same laboratory (Laboratory of Plant Breeding, Wageningen University), 271 AFLP markers were polymorphic in at least two of these populations. Only 3 out of 1,362 AFLP markers mapped in the three populations at Wageningen University were unambiguously in common with the 594 AFLP markers mapped in the OWB population at the Oregon State University. The barley populations developed at Wageningen shared common parental lines (Vada or SusPtrit) and were genotyped with at least 17 identical primer combinations while the OWB population had no parental line in common with the other populations and was genotyped with only 8 primer combinations identical to one of the three other AFLP maps. This can only partly explain the near absence of common AFLP markers identified between

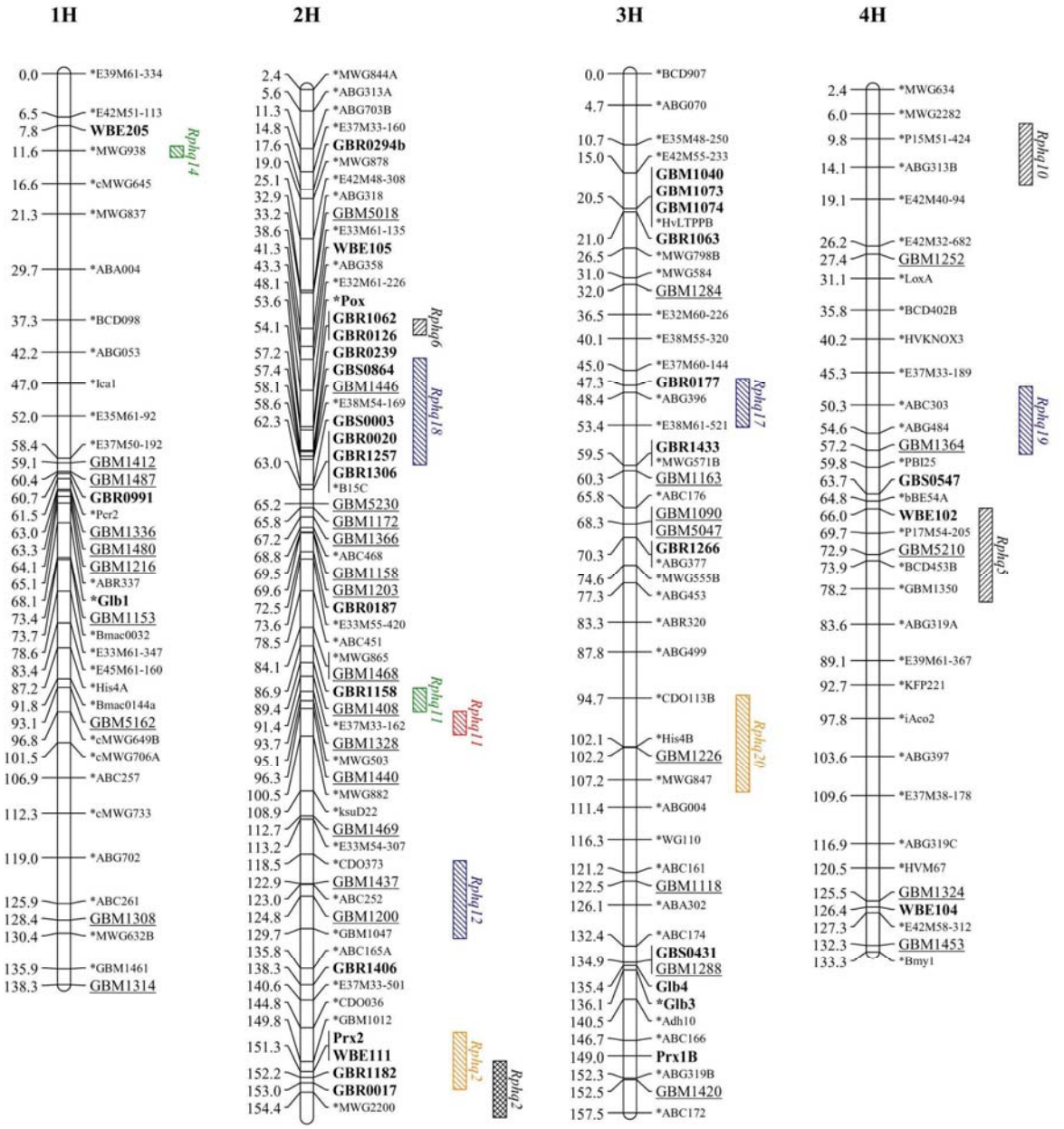
the maps developed in different laboratories. We assume that differences in the assessment of fragment sizes of AFLP bands by different laboratories are mostly responsible for the lack of common markers identified. Differences in assessed sizes could result from the use of a different visualisation system, size ladder or scoring methodology. The generation of reference AFLP fingerprints including parental lines from the populations involved and making them publicly accessible can further enhance the identification of common markers between unrelated barley mapping populations studied at different laboratories.

The lack of polymorphism observed on chromosome 6H of the St × M map over 30 cM may be due to sharing a common ancestor by the two parents. The pedigree analysis of Steptoe and of Morex revealed that they share five barley lines in their ancestry: Eckendorfer, Frew. Berg, Schladener I, Schwarze and Titan. We presume that the lack of polymorphism on 6HL is indeed due to shared ancestry.

Approximately 75% of the consensus map was associated with regions of skewed segregation in one or more of the six integrated populations. In this study, no difference was observed between the skewness of marker segregation from the DH and from the RIL populations, i.e. respectively 24 and 22% of markers showing skewed segregation. This does not support the observation of Xu et al. (1997) who reported significantly higher frequencies of skewed markers in RIL populations than in other population structures. Skewed segregation may arise from genetic, physiological and/or environmental causes and the relative contribution of each of these factors may depend on parental combination and factors during the development of the mapping population (reviewed in Xu et al. 1997).

Optimising the mapping of gene-targeted markers in plants

The sequence data generated by large-scale EST projects has made it feasible to develop molecular markers directly from genes rather than from anonymous DNA fragments. The development of gene-targeted markers (GTM) (Andersen and Lübberstedt 2003) is particularly relevant in plant species like barley for which genome sequencing cannot be completed at short term and for which a large number of ESTs is available. The ongoing development of genetic maps based on GTMs, also called transcript maps, in barley by Sato et al. (2004), Rostoks et al. (2005) and Stein et al. (2007) has already produced sets of 1,055 (SNP), 323 (SNP, SSR) and 1,032 (RFLP, SNP, SSR) GTMs, respectively. In this study, we contributed 75 new GTMs to the barley community, 61 SSRs (GBM-markers) and 14 CAPS and SCAR markers (WBE-markers), which will serve to improve the available barley transcript maps. The conversion of expressed sequence information into molecular markers with a position on a linkage map is a laborious and costly process. In order to minimise the effort and to avoid the mapping of redundant ESTs from one laboratory to another it would be advisable to integrate all contributions on one public transcript map. The construction of such



a high-density consensus barley linkage map, integrating the individual linkage maps used to map GTMs, could be achieved with the methodology that we applied in this paper.

Among the different marker technologies available to develop GTMs, genic SSRs have proven as markers of choice for their high quality and the robustness of their amplification patterns along with their multiallelic nature, codominant inheritance and superiority in terms of transferability and comparative mapping in related species (Varshney et al. 2005a; Parida et al. 2006). Nevertheless, SSR markers often produce a complex mixture of PCR products that requires high-resolution separation on polyacrylamide gels. The direct synthesis of a fluorescently labelled primer is about five times more expensive than the synthesis of an unlabelled primer. The use of tailed primer labelling to label PCR product also

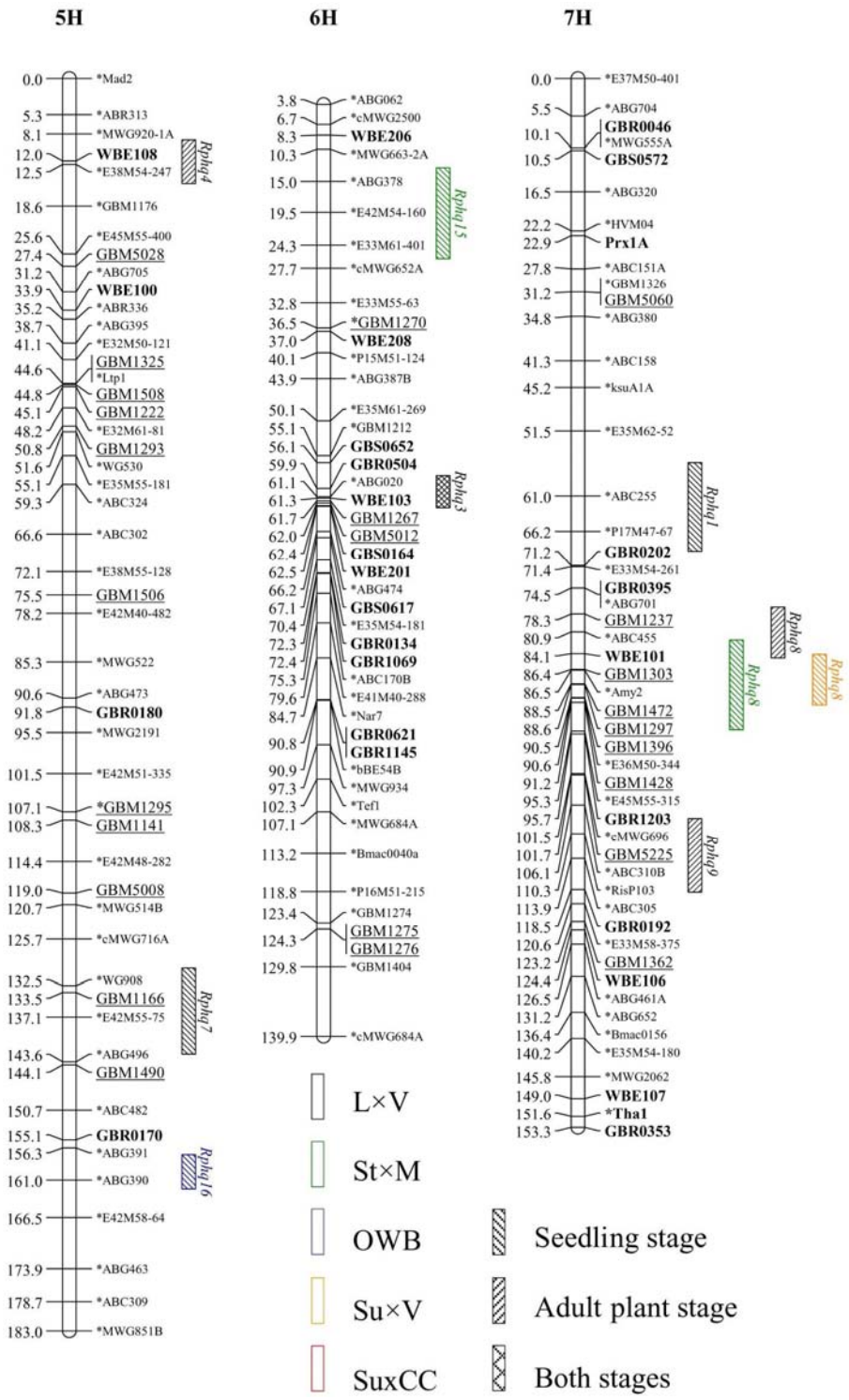


Figure 3. Locations of 19 QTLs for partial resistance to barley leaf rust mapped in five individual barley linkage maps and of 63 defence gene homologues (DGH) on the BIN map extracted from the constructed high-density consensus map of barley. Length of QTL bars corresponds approximately to the two LOD support intervals (from peak marker) based on the results of MQM. The loci preceded by an *asterisk* are BIN markers, the 61 loci *underlined* are new genic SSRs and the 63 loci in *bold* are DGH-based markers. Numbers on the left side show the distance in centiMorgans (according to Kosambi) from the top of each chromosome. The full consensus map is available as an Excel file in Table ESM S3.

results in extra costs due to the elongated size of the tailed primers and to the requirement of a second PCR. The economic aspect becomes especially relevant when a large number of primer combinations has to be tested on a small number of individuals. In this study, 313 unlabelled primer combinations were screened between the parents of two mapping populations and 61 new genic SSR markers were mapped. We optimised this extensive SSR analysis by fluorescent labelling of unlabelled PCR-mixtures followed by size-separation on

polyacrylamide gels. A procedure referred to as A/T labelling. Subsequently, the primer combinations amplifying markers of interest for high throughput applications can be directly synthesised with a fluorescent label. Automated sequencers are widely used for DNA sequencing, SSR analysis, AFLP analysis and reverse genetics. These sequencers are perfectly suited for the high-resolution size separation, detection and analysis of PCR products. We presume that the A/T labelling procedure can also be applied with other fluorescent dyes.

Distribution of QTLs for partial resistance to leaf rust on the barley genome

The level of partial resistance to leaf rust among spring barley germplasm is not only high but also increasing due to selection against high levels of susceptibility by the breeders (Niks et al. 2000b). The continued increase of levels of partial resistance in modern barley germplasm implies that there is an abundance of loci carrying such genes. The present study supports this assumption. Each parental barley combination segregates for different sets of QTLs, with only few QTLs shared by any pair of cultivars. In total, 19 QTLs were placed on the present barley consensus map. Those results confirm the earlier observations of Qi et al. (2000) and show that the abundance of QTLs for partial resistance is a reality.

Significance of the candidate gene analysis for cloning a QTL

The main challenge of GTMs development is to associate sequence polymorphisms with phenotypic variation. Several authors already mapped QTLs on linkage maps that contain GTMs (Chen et al. 2001; Faville et al. 2004; Pajerowska et al. 2005). This may allow the identification of associations between markers that are based on genes with known or putative function and QTLs for agronomic traits. The candidate gene approach has often been used to characterise disease resistance loci. Numerous genes involved in pathogen recognition, signal transduction and defence have been isolated. Traditionally, analogues of those resistance (RGA) or defence genes (DGA) are used to identify candidate genes (Pflieger et al. 2001; Lanaud et al. 2004). More recently, a procedure based on the selection of homologues of genes involved in plant defence by blast analysis was applied to identify candidate genes (Pajerowska et al. 2005). We propose to name those genes Defence Gene Homologues (DGH). In this study, eight BINs were co-occupied by a QTL and by a DGH(s) involving genes that encoded receptor-like kinase (RLK), *WIR1* homologues and several defence response genes like peroxidases, superoxide dismutase and thaumatin. Those results indicate striking similarities with previous reports, where genes with such functions also tended to co-localise with QTLs for disease resistance in wheat and in rice (Faris et al. 1999; Wang et al. 2001; Ramalingam et al. 2003). In wheat, the *WIR1* gene has a function in increasing the adhesion of the membrane to the cell wall in case of pathogen attack (Bull et al. 1992). In

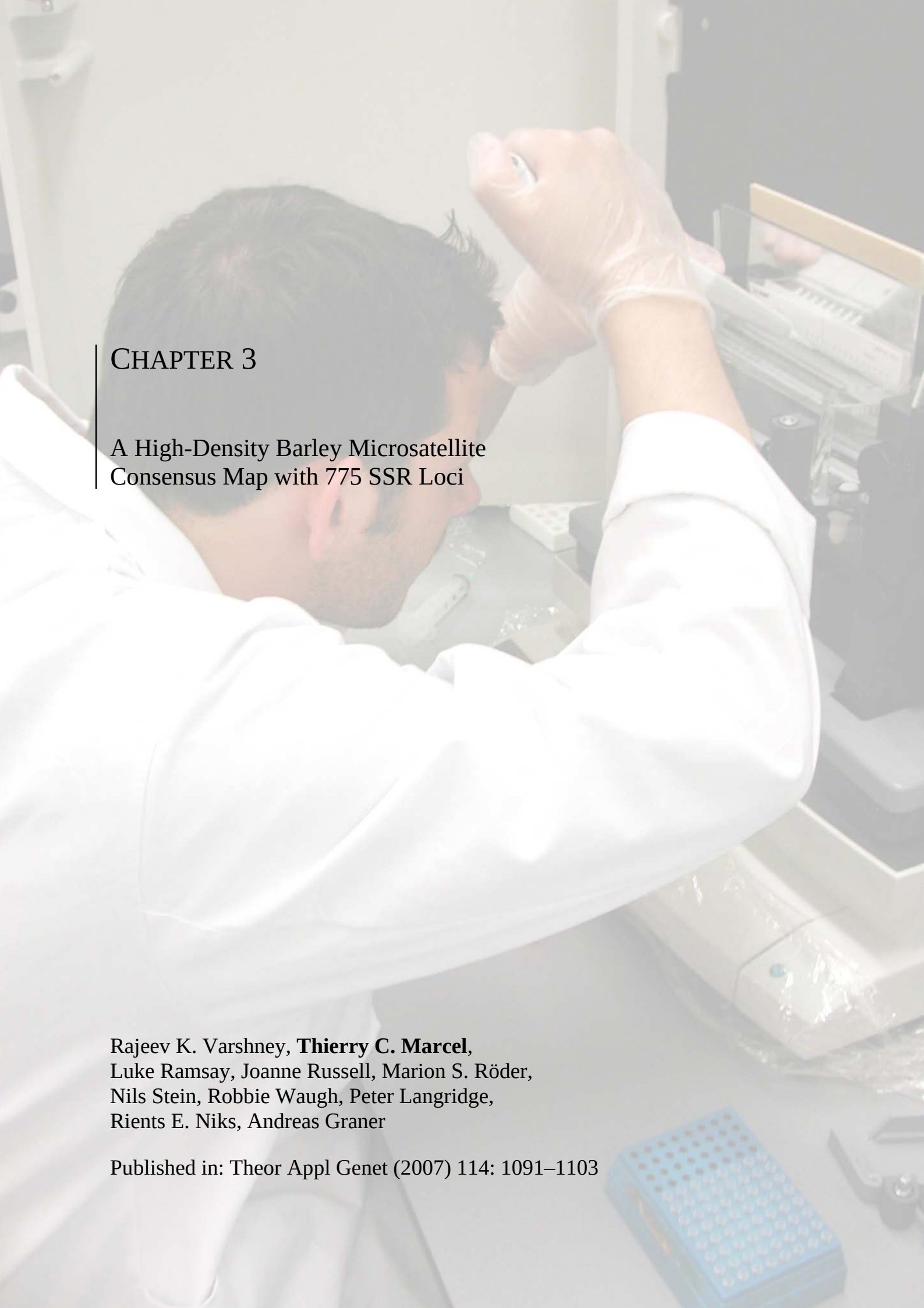
barley, *WIR1* and *WIR1* homologues were induced upon inoculation with the host pathogen *Blumeria graminis* f. sp. *hordei* (Jansen et al. 2005; Zierold et al. 2005) and with the non-host pathogen *P. triticina* (Neu et al. 2003). It is often assumed that DR genes like those encoding peroxidase (PR-9), superoxide dismutase and thaumatin-like protein (PR-5) are potential candidates to explain the QTLs for quantitative resistance to plant pathogens. Peroxidase (H_2O_2) and superoxide dismutase (O_2^-) are reactive oxygen intermediates (ROIs). ROIs have been implicated in signal transduction as well as in the execution of defence reactions such as cell wall strengthening and a rapid hypersensitive reaction (reviewed in Hückelhoven and Kogel 2003). But the role of ROIs in the establishment and maintenance of either host cell inaccessibility or accessibility during attack by a fungal plant pathogen is not yet fully understood. The vacuolar peroxidase *Prx7* was implicated as a susceptibility factor in the response of barley to attack by *B. graminis* f. sp. *hordei*, enhancing successful haustorium formation (Kristensen et al. 2001). *Prx7* mapped in the same region of chromosome 2HL (Giese et al. 1993) as *Prx2*, which is another peroxidase gene locus identified as a candidate to explain *Rphq2* in this study. The mildew haustorium promoting effect of *Prx7* (Kristensen et al. 2001) qualifies peroxidase genes as candidates for QTLs for partial resistance to *P. hordei*.

However, it always remains to be determined whether the candidate gene and the QTL map in the same position on the linkage map by chance or indeed because the candidate gene really is responsible for the phenotype determined by the QTL. For instance, many of the mapped barley DGHs were organised in clusters composed of homologous genes. DR gene families are often organised in complex loci as described by Muthukrishnan et al. (2001). So the fact that a DGH is co-segregating with a QTL does not mean that this DGH is the gene underlying the QTL. Remarkably, a cluster of DGHs mapped in the centromeric region of chromosome 6H was composed of homologues of genes from very different families like At4g22240, pBI-1, Sod, HvNR-F6 and PAL. This region of chromosome 6H might represent a gene rich region. We also performed a Chi-square test, which showed that the distribution of the 19 QTLs for partial resistance to barley leaf rust was significantly associated with the distribution of the 63 DGHs mapped on the present consensus map. It implies a tendency of QTLs and DGHs to co-segregate, and might suggest that some of the co-segregating DGHs are indeed responsible for the phenotype determined by the QTLs. At the end, fine-mapping experiments are necessary to locate precisely the implicated candidate gene and the QTL locus. Transcriptome profiling can confirm the involvement of the gene in the biochemical pathway leading to the phenotype observed but will not demonstrate conclusively whether the candidate gene is the gene determining the trait variation in the mapping population. The evidence that a candidate gene is really responsible for the trait variation can be definitively demonstrated by genetic transformation experiments.

Note

Tables ESM are available with the online version of the article (DOI: 10.1007/s00122-006-0448-2). All the mapping data and segregation data of the three RIL populations, L94 × Vada, SusPtrit × Vada and SusPtrit × Cebada Capa, used to construct the high-density consensus map of barley, have been deposited in the GrainGenes 2.0 database.

Acknowledgments Dr. Nils Stein and Dr. Marion Röder of the Institute of Plant Genetics and Crop Plant Research (IPK) are gratefully acknowledged for providing primer sequences of GBM- and GBMS-microsatellite markers. Dr. Patrick Hayes of the Oregon State University has sent us seeds of the 150 DH lines of the Steptoe × Morex population, which we greatly appreciate. We thank Dr. Patrick Schweizer of IPK for the gene expression analysis, which permitted to identify defence genes in L94. We thank Minh Truong Ta and Mumta Chhetri for assistance in mapping QTLs in the Steptoe × Morex population. We thank Benoit Gorguet, Fabien Marcel, Vincent Hennette, Simon Duquesne, Giorgia Albertazzi and Gonzalo Remiro Rodenas for marker analyses. We thank Dr. Joao Paulo for her help with the statistical analysis. We are grateful to Anton Vels of Unifarm, Wageningen University, for his technical support. This project was sponsored by the Dutch Organisation for Scientific Research (NWO Aard- en Levenswetenschappen, project no. 809.36.001).



CHAPTER 3

A High-Density Barley Microsatellite Consensus Map with 775 SSR Loci

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A student is loading a LICOR 4200 DNA sequencer

A high-density barley microsatellite consensus map with 775 SSR loci

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Abstract A microsatellite or simple sequence repeat (SSR) consensus map of barley was constructed by joining six independent genetic maps based on the mapping populations 'Igri × Franka', 'Steptoe × Morex', 'OWBRec × OWBDom', 'Lina × Canada Park', 'L94 × Vada' and 'SusPtrit × Vada'. Segregation data for microsatellite markers from different research groups including SCRI (Bmac, Bmag, EBmac, EBmag, HV*GeneName*, scsssr), IPK (GBM, GBMS), WUR (GBM), Virginia Polytechnic Institute (HVM), and MPI for Plant Breeding (HV*GeneName*), generated in above mapping populations, were used in the computer program RECORD to order the markers of the individual linkage data sets. Subsequently, a framework map was constructed for each chromosome by integrating the 496 "bridge markers" common to two or more individual maps with the help of the computer programme JoinMap® 3.0. The final map was calculated by following a "neighbours" map approach. The integrated map contained 775 unique microsatellite loci, from 688 primer pairs, ranging from 93 (6H) to 132 (2H) and with an average of 111 markers per linkage group. The genomic DNA-derived SSR marker loci had a higher polymorphism information content value (average 0.61) as compared to the EST/gene-derived SSR loci (average 0.48). The consensus map spans 1,068 cM providing an average density of one SSR marker every 1.38 cM. Such a high-density consensus SSR map provides barley molecular breeding programmes with a better choice regarding the quality of markers and a higher probability of polymorphic markers in an important chromosomal interval. This map also offers the possibilities of thorough alignment for the (future) physical map and implementation in haplotype diversity studies of barley.

Additionnal keywords: molecular markers; genetic map; EST-SSRs; barley; consensus map

Introduction

Molecular genetic maps of crop species find a variety of uses not only in breeding but also in genomics research. For instance, molecular genetic maps have been extensively used for comparative genomic studies, throwing light on genome organisation in grasses in general and in cereal crops in particular. Molecular genetic maps are also used for the identification and mapping of genes and quantitative trait loci (QTLs) for morphological, physiological and economic traits of crop species.

In barley, the first molecular genetic maps comprised RFLP markers (Graner et al. 1991; Kleinhofs et al. 1993) and over time, PCR based molecular markers became the dominant marker type (see Varshney et al. 2004). Among different types of molecular markers available for barley, microsatellite or simple sequence repeats (SSRs) have proven to be the markers of choice for marker-assisted selection (MAS) in breeding and genetic diversity studies. This is largely because they require small amounts of sample DNA, are easy to detect by PCR, are amenable to high-throughput analysis, co-dominantly inherited, multi-allelic, highly informative and abundant in genomes (Powell et al. 1996; Gupta and Varshney 2000). The value of microsatellite markers for both genetic diversity studies and for barley breeding was demonstrated as early as 1994 (Saghai Maroof et al. 1994; Becker and Heun 1995; Liu et al. 1996; Struss and Plieske 1998). Later, comprehensive microsatellite genetic maps integrating 242 SSR loci and 127 SSR loci were prepared by Ramsay et al. (2000) and by Li et al. (2003), respectively. In the majority of the studies mentioned above, the SSR markers were developed after screening small insert or microsatellite enriched genomic libraries for SSR motifs. In recent years, however, because of the availability of large expressed sequence tag (EST) datasets for a number of plant species and the development of several bioinformatics tools, it has been possible to identify and develop SSR markers from ESTs (Pillen et al. 2000; Thiel et al. 2003; Ramsay et al. 2004; Varshney et al. 2006a). The SSR markers derived from ESTs are commonly known as “EST-SSRs”. The development of such markers, in contrast to the earlier genomic SSRs, is easier, faster and cheaper (Varshney et al. 2005a).

Ideally, a molecular genetic map should be densely populated with PCR-based markers. This is especially important as barley genomics research increasingly involves map-based gene cloning projects that require accurate, fine genetic maps to correctly position a gene of interest between closely linked flanking markers (Stein and Graner 2004). To further facilitate such studies, efforts are currently underway to prepare sub-genomic physical maps with the eventual objective of capturing and sequencing the barley gene-space (http://phymap.ucdavis.edu:8080/barley/index.jsp).

Although several hundreds of microsatellite markers have been developed, they have been mapped in several mapping populations that vary in their level of polymorphism

(Varshney et al. 2004). To increase the density of microsatellite markers available on the overall barley genetic map and to provide relative locations, the present study aimed to construct a consensus genetic map integrating all available SSR-marker data. This goal was achieved by employing common markers (RFLP, AFLP and SSR) on each chromosome to anchor the chromosome maps from different populations. The final consensus map included 775 microsatellite marker loci offering a significant improvement over any single population genetic map. The distribution of different types of SSR loci and the PIC values for the markers are discussed.

Materials and methods

Mapping populations

A total of six mapping populations were integrated into a single consensus map. These included two recombinant inbred line (RIL) populations and four doubled haploid (DH) populations (Table 1). The RIL populations have been developed at the Laboratory of Plant Breeding, Wageningen University, The Netherlands, and consist of L94 × Vada (L × V) developed by Qi et al. (1998a) and of SusPtrit × Vada (Su × V) developed by Jafary et al. (2006a). The two DH populations Steptoe × Morex (St × M) and the Oregon Wolfe Barleys (OWBs), developed in North America, are reference mapping populations and subjects of extensive genotyping and phenotyping. The St × M population is the product of the North American Barley Genome Mapping Project (NABGMP) (Kleinhofs et al. 1993) and the OWB population was developed by Costa et al. (2001). The Igri × Franka DH population (I × F) was developed by Graner et al. (1991). The Lina × *Hordeum spontaneum* Canada Park (Li × Hs) is a DH population from Svalof Weibull and was used by SCRI (Ramsay et al. 2000) to genetically map 242 SSR marker loci.

SSR markers and segregation data

Several sources of SSR markers, listed in Table 2, and mapped in different mapping populations were used to prepare the barley microsatellite consensus map. These markers included both marker types, derived from genomic DNA as well as from genes or ESTs. More than ten designations have been assigned to these markers by the laboratory that developed the markers (Table 2, Tables ESM S1, S2).

The segregation data of 968 marker loci mapped in L × V and of 450 marker loci mapped in Su × V were obtained from Marcel et al. (2007). Those data sets predominantly consisted of AFLP markers, but also included 138 and 24 microsatellite loci, respectively (Table 1). Two barley segregation data sets were downloaded from the publicly available

Table 1. Summary of individual mapping data used to construct the microsatellite consensus map of barley

Population number	Name of the mapping population	Type of population	Number of lines	Total number of markers	Predominant marker type	Number of SSR markers	Number of SSR markers in common with *n* other mapping populations				
							n=0	n=1	n=2	n=3	n=4
1	L94 × Vada (L × V)	F_9 RIL[1]	103	968	AFLP[3]	138	57	38	19	17	7
2	SusPtrit × Vada (Su × V)	F_8 RIL	152	450	AFLP	24	12	0	2	8	2
3	Steptoe × Morex (St × M)	DH[2]	150	694	RFLP[4]	218	110	70	17	15	6
4	OWB_{Rec} × OWB_{Dom} (OWB)	DH	94	995	AFLP	230	156	34	17	16	7
5	Igri × Franka (I × F)	DH	71	695	RFLP	139	54	60	10	9	6
6	Lina × *H. spontaneum* (Li × Hs)	DH	84	418	SSR[5]	307	195	68	22	15	7
	Total						***584***	***135***	***29***	***20***	***7***

[1] Recombinant Inbred Line

[2] Doubled Haploid

[3] Amplified Fragment Length Polymorphism

[4] Restriction Fragment Length Polymorphism

[5] Simple Sequence Repeat

GrainGenes 2.0 database (http://wheat.pw.usda.gov/GG2/index.shtml), for the St × M and I × F populations, respectively. Those two data sets predominantly consisted of RFLP markers, to which the segregation data for 218 and 139 microsatellite loci (Table 1) were added, respectively. Another set of segregation data was downloaded from the Oregon State University (OSU) Barley Project web site (http://www.barleyworld.org/), for the OWBs. Most of the markers mapped in the OWB population are AFLP markers, but the segregation data for 230 microsatellite loci could also be obtained (Table 1). Within the latter set of 230 microsatellite loci, 34 are new scssr (SCRI-SSR) loci recently integrated into a SNP map of barley (Rostoks et al. 2005) and provided by Joanne Russell. Finally, the segregation data of 418 marker loci, 307 being microsatellite loci, mapped in Li × Hs were provided by Luke Ramsay (Table 1).

The genotyping data for all the SSR loci mapped in different mapping populations have been appended as Table ESM S3.

Marker ordering in the individual maps

The recently developed computer program RECORD (Van Os et al. 2005a) was used to order the markers from the six individual linkage data sets, which comprised from 400 to 1,000 markers per set (Table 1). RECORD employs a marker-ordering algorithm based on minimisation of the total number of recombination events in any given marker order. The linkage groups were sorted by graphical genotyping in Microsoft® Office Excel 2003. The ordering of markers with RECORD was repeated three times for each individual linkage map. Between each two marker orderings, singletons and other potential errors in the marker segregation data were identified by visual inspection of graphical genotypes. The identified singletons (a single locus in one progeny line that appears to have recombined with both its directly neighbouring loci) were replaced by missing values as suggested by Isidore et al. (2003) and Van Os et al. (2005b).

Production of the framework map

The RECORD software package does not offer the possibility to integrate different marker data sets. The integration module of the software package JoinMap® 3.0 (Van Ooijen and Voorrips 2001) could also not be used directly because it cannot handle sets of several thousands of segregating markers. Then, the integrative function of JoinMap® 3.0 was used to construct a framework map for each chromosome containing only the bridge markers identified between two or more populations. A bridge marker was considered as such when it had an (almost) identical name and a similar map position in the different mapping populations concerned. Markers with the same name that mapped to different positions in different populations were not considered to be common. The obtained framework maps

contained 45, 86, 82, 54, 69, 68 and 79 integrated bridge markers for the barley linkage groups 1H to 7H, respectively. Those 496 bridge markers consist of 191 SSRs, 160 AFLPs, 139 RFLPs and 6 genes mapped by function spanning 1,024 cM with an average density of one marker every 2.1 cM. All markers were assigned to a chromosome during the marker ordering procedure. For each chromosome, the identified bridge markers were assembled and the corresponding framework map calculated separately in JoinMap® 3.0. The values used to calculate the maps ranged from 0.2 to 1.0 for the LOD (logarithm of odds) threshold and from 0.400 to 0.490 for the recombination threshold, depending on the linkage group. The map distances were calculated using the Kosambi mapping function.

Table 2. Details on microsatellite loci integrated into the consensus map

Microsatellite code	Source of markers	Number of loci	Developing laboratory	References
AF, BAC	BAC end sequences	4	SCRI (R. Waugh)	Ramsay et al. 2000, Cardle et al. 2000
Bmac, EBmac	Genomic DNA libraries (AC repeats)	157	SCRI (R. Waugh)	Ramsay et al. 2000
Bmag, EBmag	Genomic DNA libraries (AG repeats)	135	SCRI (R. Waugh)	Ramsay et al. 2000
Bmg	Genomic DNA library	2	SCRI (R. Waugh)	Ramsay et al. 2000
EBmatc	Genomic DNA library (ATC repeats)	6	SCRI (R. Waugh)	Ramsay et al. 2000
GMS	Genomic DNA libraries (GA and GT repeats)	12	IPK (D. Struss)	Struss and Plieske 1998, Li et al. 2003
GBMS	Genomic DNA libraries (GA and GT repeats)	119	IPK (M. Röder, M. Ganal)	Li et al. 2003
HVM	Majority from genomic DNA and some from genes	34	VPISU (M.A. Saghai Maroof)	Saghai Maroof et al. 1994, Liu et al. 1996, Li et al. 2003
HV*GeneName*	Barley genes	7	MPIZ (M. Heun)	Becker and Heun 1995
HV*EMBLName*	Barley genes	17	SCRI (R. Waugh), Univ Bonn (K. Pillen)	Ramsay et al. 2000 Pillen et al. 2000
GBM	Barley ESTs	246	IPK (A. Graner), WUR (R.E. Niks)	Thiel et al. 2003, Varshney et al. 2006a Marcel et al. 2007
scssr	Barley ESTs	34	SCRI (R. Waugh)	Ramsay et al. 2004, Rostoks et al. 2005
WM	Wheat microsatellites from genomic DNA libraries	2	SCRI (R. Waugh), VPISU (M.A. Saghai Maroof)	Ramsay et al. 2000 Liu et al. 1996

Construction of the SSR consensus map

The final map comprising all 3,610 markers was calculated based on the "neighbours" map approach described by Cone et al. (2002). A new improved version of JoinMap based on a faster algorithm (Jansen et al. 2001) was kindly provided by Dr. van Ooyen (www.kyazma.nl). The six individual barley maps were recalculated by adding the order of the framework markers, as given by JoinMap® 3.0, as a "fixed order file" into this improved version of JoinMap. Then, the framework map served as a fixed backbone onto which the unique loci of each newly calculated individual map were added. For a target locus, the two nearest flanking bridge markers shared by the framework map and by the map to integrate were identified and the coordinate of this locus was calculated relative to the ratio of the intervals defined by the flanking bridge markers on the two maps. In such a way, an integrated map of 3,610 markers was obtained from which the coordinates of 775 unique microsatellite loci were extracted. In the final microsatellite integrated map of barley the position of BIN markers, as defined by Marcel et al. (2007), are given as reference. Mostly, the same BIN-defining markers and numbers as defined by Kleinhofs and Graner (2001) were maintained. Each 10 cM BIN was subdivided into two 5 cM sub-BINs.

Polymorphism Information content (PIC)

The PIC is a tool to measure the informativeness of a given DNA marker. The PIC value is generally calculated using the following formula (Anderson et al. 1993).

$$PIC = 1 - \sum_{i=1}^{k} P_i^2$$

where *k* is the total number of alleles detected for a microsatellite and *Pi* the frequency of the *i*th allele in germplasm investigated.

The PIC value for the SSR markers developed at IPK and WUR was calculated using the above formula. However, the PIC value for a majority of the markers integrated into the microsatellite consensus map was taken from the original publications in which the corresponding markers were first reported (Table 2). Other publications reported PIC values of SSR markers calculated on different sets of barley lines and cultivars (Matus and Hayes 2002; Ivandic et al. 2003; Karakousis et al. 2003; Sjakste et al. 2003; Malysheva-Otto et al. 2006). Those PIC values were compiled in Microsoft® Office Excel and identical microsatellites (identical name) between marker sets were identified and aligned. For each set of values, microsatellites in common with the ones reported in this paper were used to calculate a correlation coefficient.

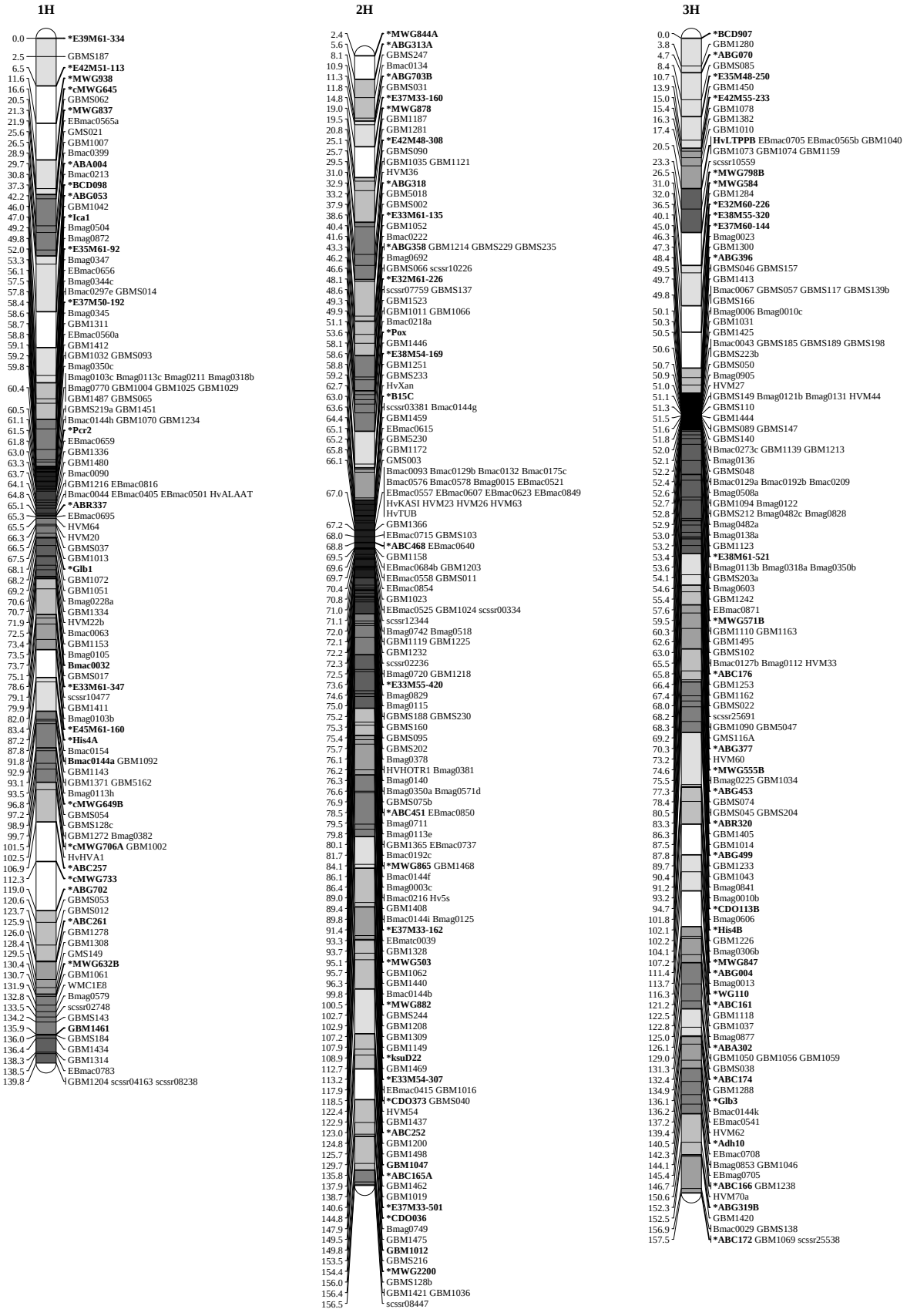
Results

Consensus microsatellite map

The present barley microsatellite consensus map contains a total of 775 microsatellite loci mapped with 688 microsatellite primer combinations in one or more of the six barley populations used (Fig. 1, Tables ESM S1, S2). In total 191 SSR markers were in common, i.e. they were mapped in at least two mapping populations (Table 1). A total of 584 SSR marker loci were mapped only once in a particular mapping population, while seven SSR marker loci were mapped in five mapping populations. The RECORD order of those markers that segregated in more than one population was highly consistent between the six individual mapping data sets. On the consensus map, linkage group 2H had the highest number of markers (132) with an average marker density 1/1.19 cM followed by linkage group 7H (127) with an average marker density 1/1.24 cM (Table 3). Linkage group 6H had the smallest number of markers (93) and the lowest marker density (1/1.75 cM) was observed on linkage group 5H. Although all linkage groups had a more or less uniform distribution of SSR loci, some gaps of 14–22 cM without microsatellite marker were observed on the distal ends of linkage groups 5H and 6H (Fig. 1). Clustering of microsatellite markers at centromeric regions was observed with 33.5% of the markers found in 5.6% of the BINs. In total, the consensus microsatellite map of barley had 1,068 cM genome coverage with an average density of one microsatellite per 1.38 cM. The BIN marker order of the present consensus map was inspected for inconsistencies with the order of the same markers on the BIN map of Kleinhofs and Graner (2001) and of the Steptoe × Morex and Igri × Franka linkage maps. The marker orders between the maps were in good agreement with only two inversions of markers on chromosome 3HS and at the distal end of chromosome 5HL. Chromosomes 3HS and 5HL were recalculated by adding the BIN markers of Kleinhofs and Graner (2001) as fixed order in JoinMap® 3.0. The present SSR consensus map was also aligned with the SSR maps developed by Ramsay et al. (2000) (GrainGenes: “Barley, L×Hs”) and by Li et al. (2003) (GrainGenes: “Barley, Steptoe × Morex, SSR”). The SSR marker orders were highly consistent between all maps. Nevertheless, differences in the order of markers were observed within the centromeric BINs of the linkage groups from the present consensus map and from the map of Ramsay et al. (2000). The primer sequences for the SSR loci integrated into the consensus map, wherever possible, are given in Table ESM S2 and the genotyping data for all the SSR loci are given in Table ESM S3.

Nomenclature of SSR loci

Several SSR developing laboratories have designated their SSR markers by their own codes (or code systems) (Table 2). The SSR markers that mapped in more than one mapping popula-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16.6 *cMWG645
20.5 GBMS062
21.3 *MWG837
21.9 EBmac0565a
25.6 GMS021
26.5 GBM1007
28.9 Bmac0399
29.7 *ABA004
30.8 Bmac0213
37.3 *BCD098
42.2 *ABG053
46.0 GBM1042
47.0 *Ica1
49.2 Bmag0504
49.8 Bmag0872
52.0 *E35M61-92
53.3 Bmag0347
56.1 EBmac0656
57.5 Bmag0344c
57.8 Bmac0297e GBMS014
58.4 *E37M50-192
58.6 Bmag0345
58.7 GBM1311
58.8 EBmac0560a
59.1 GBM1412
59.2 GBM1032 GBMS093
59.8 Bmag0350c
Bmag0103c Bmag0113c Bmag0211 Bmag0318b
60.4 Bmag0770 GBM1004 GBM1025 GBM1029
GBM1487 GBMS065
60.5 GBMS219a GBM1451
61.1 Bmac0144h GBM1070 GBM1234
61.5 *Pcr2
61.8 EBmac0659
63.0 GBM1336
63.3 GBM1480
63.7 Bmac0090
64.1 GBM1216 EBmac0816
64.8 Bmac0044 EBmac0405 EBmac0501 HvALAAT
65.1 *ABR337
65.3 EBmac0695
65.5 HVM64
66.3 HVM20
66.5 GBMS037
67.5 GBM1013
68.1 *Glb1
68.2 GBM1072
69.2 GBM1051
70.6 Bmag0228a
70.7 GBM1334
71.9 HVM22b
72.5 Bmac0063
73.4 GBM1153
73.5 Bmag0105
73.7 Bmac0032
75.1 GBMS017
78.6 *E33M61-347
79.1 scssr10477
79.9 GBM1411
82.0 Bmag0103b
83.4 *E45M61-160
87.2 *His4A
87.8 Bmac0154
91.8 Bmac0144a GBM1092
92.9 GBM1143
93.1 GBM1371 GBM5162
93.5 Bmag0113h
96.8 *cMWG649B
97.2 GBMS054
98.9 GBMS128c
99.7 GBM1272 Bmag0382
101.5 *cMWG706A GBM1002
102.5 HvHVA1
106.9 *ABC257
112.3 *cMWG733
119.0 *ABG702
120.6 GBMS053
123.7 GBMS012
125.9 *ABC261
126.0 GBM1278
128.4 GBM1308
129.5 GMS149
130.4 *MWG632B
130.7 GBM1061
131.9 WMC1E8
132.8 Bmag0579
133.5 scssr02748
134.2 GBMS143
135.9 GBM1461
136.0 GBMS184
136.4 GBM1434
138.3 GBM1314
138.5 EBmac0783
139.8 GBM1204 scssr04163 scssr08238
2H
2.4 *MWG844A
5.6 *ABG313A
8.1 GBMS247
10.9 Bmac0134
11.3 *ABG703B
11.8 GBMS031
14.8 *E37M33-160
19.0 *MWG878
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20.8 GBM1281
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37.9 GBMS002
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49.9 GBM1011 GBM1066
51.1 Bmac0218a
53.6 *Pox
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58.6 *E38M54-169
58.8 GBM1251
59.2 GBMS233
62.7 HvXan
63.0 *B15C
63.6 scssr03381 Bmac0144g
64.4 GBM1459
65.1 EBmac0615
65.2 GBM5230
65.8 GBM1172
66.1 GMS003
Bmac0093 Bmac0129b Bmac0132 Bmac0175c
Bmac0576 Bmac0578 Bmag0015 EBmac0521
67.0 EBmac0557 EBmac0607 EBmac0623 EBmac0849
HvKASI HVM23 HVM26 HVM63
HvTUB
67.2 GBM1366
68.0 EBmac0715 GBMS103
68.8 *ABC468 EBmac0640
69.5 GBM1158
69.6 EBmac0684b GBM1203
69.7 EBmac0558 GBMS011
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70.8 GBM1023
71.0 EBmac0525 GBM1024 scssr00334
71.1 scssr12344
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72.2 GBM1232
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72.5 Bmag0720 GBM1218
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75.2 GBMS188 GBMS230
75.3 GBMS160
75.4 GBMS095
75.7 GBMS202
76.1 Bmag0378
76.2 HVHOTR1 Bmag0381
76.3 Bmag0140
76.6 Bmag0350a Bmag0571d
76.9 GBMS075b
78.5 *ABC451 EBmac0850
79.5 Bmag0711
79.8 Bmag0113e
80.1 GBM1365 EBmac0737
81.7 Bmac0192c
84.1 *MWG865 GBM1468
86.1 Bmac0144f
86.4 Bmag0003c
89.0 Bmac0216 Hv5s
89.4 GBM1408
89.8 Bmac0144i Bmag0125
91.4 *E37M33-162
93.3 EBmatc0039
93.7 GBM1328
95.1 *MWG503
95.7 GBM1062
96.3 GBM1440
99.8 Bmac0144b
100.5 *MWG882
102.7 GBMS244
102.9 GBM1208
107.2 GBM1309
107.9 GBM1149
108.9 *ksuD22
112.7 GBM1469
113.2 *E33M54-307
117.9 EBmac0415 GBM1016
118.5 *CDO373 GBMS040
122.4 HVM54
122.9 GBM1437
123.0 *ABC252
124.8 GBM1200
125.7 GBM1498
129.7 GBM1047
135.8 *ABC165A
137.9 GBM1462
138.7 GBM1019
140.6 *E37M33-501
144.8 *CDO036
147.9 Bmag0749
149.5 GBM1475
149.8 GBM1012
153.5 GBMS216
154.4 *MWG2200
156.0 GBMS128b
156.4 GBM1421 GBM1036
156.5 scssr08447
3H
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3.8 GBM1280
4.7 *ABG070
8.4 GBMS085
10.7 *E35M48-250
13.9 GBM1450
15.0 *E42M55-233
15.4 GBM1078
16.3 GBM1382
17.4 GBM1010
20.5 HvLTPPB EBmac0705 EBmac0565b GBM1040
GBM1073 GBM1074 GBM1159
23.3 scssr10559
26.5 *MWG798B
31.0 *MWG584
32.0 GBM1284
36.5 *E32M60-226
40.1 *E38M55-320
45.0 *E37M60-144
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47.3 GBM1300
48.4 *ABG396
49.5 GBMS046 GBMS157
49.7 GBM1413
49.8 Bmac0067 GBMS057 GBMS117 GBMS139b
GBMS166
50.1 Bmag0006 Bmag0010c
50.3 GBM1031
50.5 GBM1425
50.6 Bmac0043 GBMS185 GBMS189 GBMS198
GBMS223b
50.7 GBMS050
50.9 Bmag0905
51.0 HVM27
51.1 GBMS149 Bmag0121b Bmag0131 HVM44
51.3 GBMS110
51.5 GBM1444
51.6 GBMS089 GBMS147
51.8 GBMS140
52.0 Bmac0273c GBM1139 GBM1213
52.1 Bmag0136
52.2 GBMS048
52.4 Bmac0129a Bmac0192b Bmac0209
52.6 Bmag0508a
52.7 GBM1094 Bmag0122
52.8 GBMS212 Bmag0482c Bmag0828
52.9 Bmag0482a
53.0 Bmag0138a
53.2 GBM1123
53.4 *E38M61-521
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54.1 GBMS203a
54.6 Bmag0603
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60.3 GBM1110 GBM1163
62.6 GBM1495
63.0 GBMS102
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67.4 GBM1162
68.0 GBMS022
68.2 scssr25691
68.3 GBM1090 GBM5047
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73.2 HVM60
74.6 *MWG555B
75.5 Bmag0225 GBM1034
77.3 *ABG453
78.4 GBMS074
80.5 GBMS045 GBMS204
83.3 *ABR320
86.3 GBM1405
87.5 GBM1014
87.8 *ABG499
89.7 GBM1233
90.4 GBM1043
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93.2 Bmag0010b
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101.8 Bmag0606
102.1 *His4B
102.2 GBM1226
104.1 Bmag0306b
107.2 *MWG847
111.4 *ABG004
113.7 Bmag0013
116.3 *WG110
121.2 *ABC161
122.5 GBM1118
122.8 GBM1037
125.0 Bmag0877
126.1 *ABA302
129.0 GBM1050 GBM1056 GBM1059
131.3 GBMS038
132.4 *ABC174
134.9 GBM1288
136.1 *Glb3
136.2 Bmac0144k
137.2 EBmac0541
139.4 HVM62
140.5 *Adh10
142.3 EBmac0708
144.1 Bmag0853 GBM1046
145.4 EBmag0705
146.7 *ABC166 GBM1238
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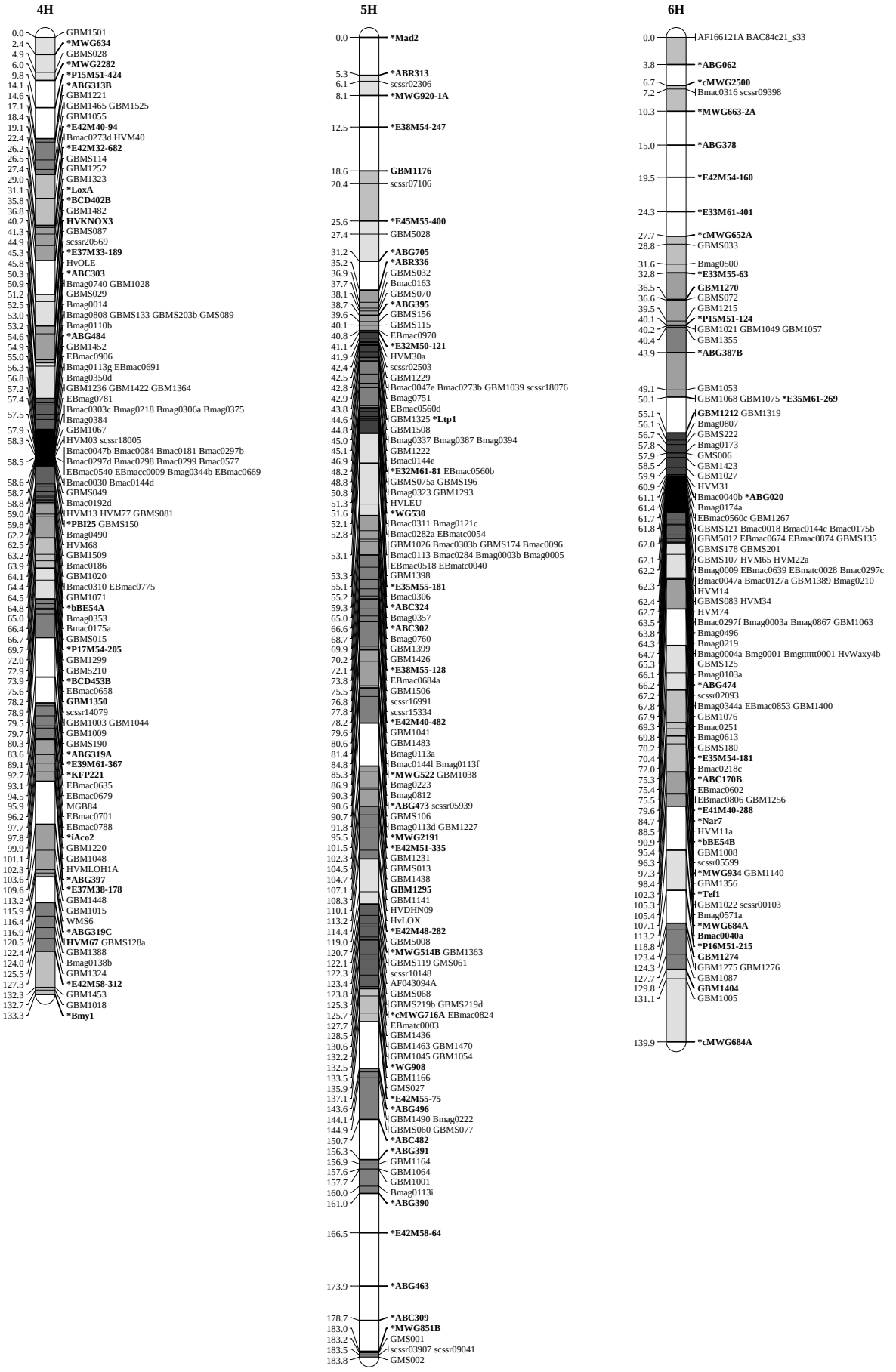
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50.3 *ABC303
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52.5 Bmag0014
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Bmag0384
57.9 GBM1067
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Bmac0297d Bmac0298 Bmac0299 Bmac0577
EBmac0540 EBmacc0009 Bmag0344b EBmac0669
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58.8 Bmac0192d
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66.4 Bmac0175a
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5H
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25.6 *E45M55-400
27.4 GBM5028
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36.9 GBMS032
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38.1 GBMS070
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Bmac0113 Bmac0284 Bmag0003b Bmag0005
EBmac0518 EBmatc0040
53.3 GBM1398
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59.3 *ABC324
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125.7 *cMWG716A EBmac0824
127.7 EBmatc0003
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130.6 GBM1463 GBM1470
132.2 GBM1045 GBM1054
132.5 *WG908
133.5 GBM1166
135.9 GMS027
137.1 *E42M55-75
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144.1 GBM1490 Bmag0222
144.9 GBMS060 GBMS077
150.7 *ABC482
156.3 *ABG391
156.9 GBM1164
157.6 GBM1064
157.7 GBM1001
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161.0 *ABG390
166.5 *E42M58-64
173.9 *ABG463
178.7 *ABC309
183.0 *MWG851B
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6.7 *cMWG2500
7.2 Bmac0316 scssr09398
10.3 *MWG663-2A
15.0 *ABG378
19.5 *E42M54-160
24.3 *E33M61-401
27.7 *cMWG652A
28.8 GBMS033
31.6 Bmag0500
32.8 *E33M55-63
36.5 GBM1270
36.6 GBMS072
39.5 GBM1215
40.1 *P15M51-124
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40.4 GBM1355
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50.1 GBM1068 GBM1075 *E35M61-269
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57.8 Bmag0173
57.9 GMS006
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61.7 EBmac0560c GBM1267
61.8 GBMS121 Bmac0018 Bmac0144c Bmac0175b
62.0 GBM5012 EBmac0674 EBmac0874 GBMS135
GBMS178 GBMS201
62.1 GBMS107 HVM65 HVM22a
62.2 Bmag0009 EBmac0639 EBmatc0028 Bmac0297c
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HVM14
62.4 GBMS083 HVM34
62.7 HVM74
63.5 Bmac0297f Bmag0003a Bmag0867 GBM1063
63.8 Bmag0496
64.3 Bmag0219
64.7 Bmag0004a Bmg0001 Bmgtttttt0001 HvWaxy4b
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66.1 Bmag0103a
66.2 *ABG474
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67.9 GBM1076
69.3 Bmac0251
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75.5 EBmac0806 GBM1256
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88.5 HVM11a
90.9 *bBE54B
95.4 GBM1008
96.3 scssr05599
97.3 *MWG934 GBM1140
98.4 GBM1356
102.3 *Tef1
105.3 GBM1022 scssr00103
105.4 Bmag0571a
107.1 *MWG684A
113.2 Bmac0040a
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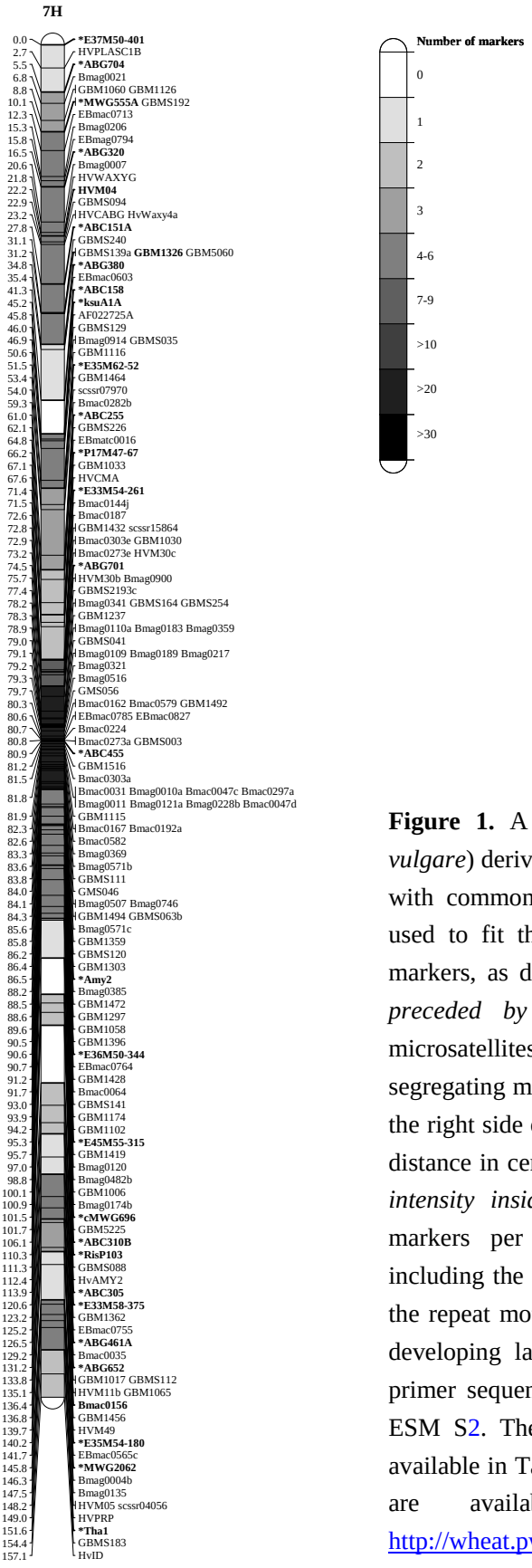

Figure 1. A microsatellite consensus map of barley (*Hordeum vulgare*) derived from six independent genetic maps. A skeleton map with common markers was constructed using JoinMap® 3.0 and used to fit the markers from the six individual maps. The BIN markers, as defined by Marcel et al. (2007) are in *bold*. The *loci preceded by an asterisk* are BIN markers, which are not microsatellites. The remaining loci are microsatellite markers. Co-segregating markers are listed next to each other in a vertical line on the right side of the chromosome. *Numbers on the left side* show the distance in centiMorgans from the top of each chromosome. *Colour intensity inside the bars* indicates the density of microsatellite markers per BIN. Detailed information about these markers including the name of microsatellite loci, the chromosome position, the repeat motif, the PIC value (if available) and the contact of the developing laboratory are available in Table ESM S1 while the primer sequences for the mapped SSR loci are available in Table ESM S2. The genotyping data for all the mapped SSR loci are available in Table ESM S3. Additionally, all the supplementary data are available at GrainGenes under the URL http://wheat.pw.usda.gov/pubs/2007/varshney/

tion are in the present study termed as common bridge markers, as these have been used to prepare the consensus map. In fact, the integration of several genetic maps depends on the number and on the distribution of common bridge markers between the individual maps. However, while checking the segregation data for markers in different mapping populations, several inconsistencies were found in the designation of the same SSR marker mapped in more than one mapping population. In order to maintain the uniformity and avoid confusion, we made some slight changes in the designations of mapped SSR loci and recommend the community to use the same in the future (Table ESM S1). For example, the Bmac, Bmag, EBmac, EBmag, EBmatc and GBM microsatellite loci were all identified with a suffix of four digits (e.g. Bmac29 becomes Bmac0029). Similarly, the GMS and GBMS microsatellite loci were identified with a suffix of three digits (e.g. GBMS2 becomes GBMS002), and the HVM microsatellite loci were identified with a suffix of two digits (e.g. HVM4 becomes HVM04). Multiple segregating bands identified with one microsatellite primer pair have been usually indicated with lower case letters; for example, two bands (loci) for the Bmac0040 SSR marker (primer pair) became Bmac0040a and Bmac0040b. However, the same letter was often assigned to different loci identified with the same microsatellite primer pair in different populations. Those markers were renamed in a way that distinctive letters were assigned to different loci (Table ESM S1).

Microsatellite repeat motifs

Out of 775 SSR loci integrated into the consensus map, information on occurrence of the SSR repeat motif was available for 768 SSR loci. More than 55% of SSR loci (435) for which repeat information was available, consisted of dinucleotide repeat motifs (NN) (Table ESM S1). Compound microsatellites occur when two different SSRs, separated by a few base pairs, are amplified with the same primer pair. In the present study, compound microsatellites consisted in a majority of NNs and were the second most common type of SSR loci (163 loci, 21%) integrated to the consensus map. The trinucleotide (NNN) and tetranucleotide (NNNN) repeat motifs were present only in 16.5% (128) and 3.6% (28) of the SSR loci, respectively. The remaining repeat classes, i.e. mononucleotide (N), pentanucleotide (NNNNN) and hexanucleotide (NNNNNN), were represented by less than 1% of the SSR loci.

Polymorphism Information Content (PIC) value

The PIC value measures the informativeness of a given DNA marker over a set of genotypes. Therefore, the PIC value of SSR markers available in a given window on the consensus map is a good indicator of their potential usefulness. For this reason, we compiled the PIC value available for the SSR markers, from the original studies, in the Table ESM S1. The PIC values

are comparable between the different sets of microsatellites because they have been calculated based on similar panels mainly composed of European breeding lines. Overall, the SSR markers that mapped on linkage group 7H had the highest average PIC value (0.59) followed by the markers mapped on linkage groups 2H and 3H. The SSR markers located on 1H had on average the lowest PIC value (0.53). The majority of SSR markers (>54%) for which a PIC value was available had a PIC value of >0.50 and about 16% of the SSR markers had a PIC value of >0.75. The genomic DNA-derived SSR marker loci had a higher PIC value (average 0.61) than the EST/gene-derived SSR loci (average 0.48) (Fig. 2).

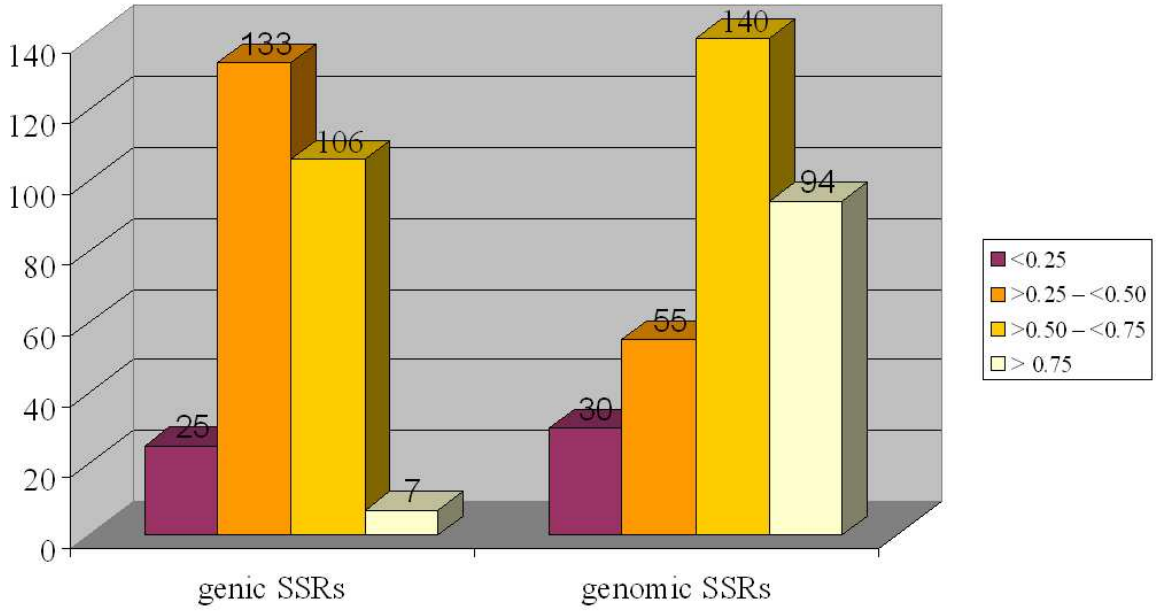

Figure 2. Distribution of the polymorphism information content (PIC) value of genic and genomic microsatellites. The markers are classified into four groups having PIC values, (1) less than 0.25, (2) between 0.25 and 0.50, (3) between 0.50 and 0.75, and (4) more than 0.75.

In general, the dinucleotide SSRs had the highest PIC value (average 0.58) as compared to mononucleotide (average 0.47), trinucleotide (average 0.46), tetranucleotide (average 0.43), pentanucleotide (average 0.50) or hexanucleotide (0.41) markers. The compound microsatellites had the highest PIC values as 0.59 (average).

The compiled PIC values for microsatellite loci of the SSR consensus map was compared to the PIC values obtained for the same microsatellite loci in other studies and on different panels of barley cultivars (Table 4). The highest correlation coefficient between PIC values (r = 0.70) was obtained with a worldwide collection of 953 accessions. About 60% of those 953 accessions are from European origin like the accessions used to calculate the PIC values compiled in our study. Lower correlation coefficients were obtained with the sets of barley breeding lines from other continents. The lowest correlation coefficient (r = 0.30) was obtained with the set of wild barley accessions (*H. spontaneum*) collected in Israel.

Functional SSR markers

Although SSR markers developed earlier were thought to be associated with retrotransposons, recent analysis on SSRs in genomic and EST sequence data have shown that microsatellite sequences also occur in genes (Morgante et al. 2002). Several gene (EST)-derived SSR markers (= genic SSR markers) have been developed in barley recently. Unlike markers derived from genomic DNA, a putative function can be deduced for gene-/EST-derived markers (Varshney et al. 2005a). Therefore, they represent a functional class of molecular markers (Andersen and Lübberstedt 2003). The functional SSR markers include earlier published genic SSR markers (Saghai Maroof et al. 1994; Becker and Heun 1995; Pillen et al. 2000) and recently developed EST-derived SSR markers (Thiel et al. 2003; Rostoks et al. 2005; Varshney et al. 2006a; Marcel et al. 2007). In total, 44% of the SSR marker loci (339) placed on the consensus map are genic/functional-SSR loci.

Table 4. Correlation coefficients between the polymorphism information content, or PIC, values compiled for the SSR loci of the consensus map, calculated on several sets of European barley cultivars, and the PIC values obtained in previous studies, calculated on different sets of barley accessions

Germplasm description	Common markers[1]	Correlation coefficient[2]	Reference
953 accessions through the world	44	0.70	Malysheva-Otto et al. 2006
37 Latvian cultivars	57	0.47	Sjakste et al. 2003
40 Australian breeding lines	55	0.43	Karakousis et al. 2003
96 North-American breeding lines	37	0.37	Matus and Hayes 2002
52 *H. spontaneum* accessions	30	0.31	Ivandic et al. 2003

[1] Number of microsatellite loci common between the SSR consensus map and the study considered for which PIC values were available

[2] Correlation coefficient between the PIC values compiled for the SSR consensus map and the PIC values obtained for the study considered

Discussion

Since the advent of molecular marker and linkage mapping technologies the number of marker loci placed on genetic maps is increasing exponentially. In crop plant species such as rice, maize and soybean, several high-density genetic maps are available (Phillips and Vasil 2001). Dense genetic maps are very useful for plant breeders to help identify molecular markers closely linked to the genes or QTLs of their interest (Varshney et al. 2006b). Further, dense genetic maps are important to prepare contig-based local or genome wide physical maps, for map-based cloning and for genome sequencing projects. Since microsatellite markers are currently preferred over other molecular markers for a variety of reasons, high

density microsatellite maps, such as those developed in rice (McCouch et al. 2002), maize (Sharopova et al. 2002) and wheat (Somers et al. 2004), are very useful.

Features of the barley SSR consensus map

Although a large number of SSR markers are available in barley, they have been developed and mapped in different mapping populations. Ideally, all markers should be mapped in the same mapping population. However, the limited polymorphism in current mapping populations has not allowed all possible SSR markers to be mapped onto a single genetic map. An alternative way to prepare a dense SSR genetic map is to combine the different and available genetic maps by exploiting common bridging markers. Consensus maps including various types of molecular markers have been developed before in several species, e.g. barley (Langridge et al. 1995; Qi et al. 1996; Karakousis et al. 2003), tomato (Haanstra et al. 1999), wheat (Somers et al. 2004), pearl millet (Qi et al. 2004) and potato (Van Os et al. 2006). We have derived the most extensive consensus SSR map of barley so far. The map displays the genetic position of microsatellites at a density (1/1.38 cM) that should enhance their application in both plant breeding and physical mapping. Despite the dense average spacing of the markers, some gaps on the distal ends of linkage groups 5H and 6H occur. These may reflect regions of high recombination. A lack of markers in these regions was observed in other genetic maps of barley (Kleinhofs et al. 1993; Qi et al. 1998a; Ramsay et al. 2000).

The consensus SSR map contains almost all types of SSR loci, however, dinucleotide and compound (mainly containing different dinucleotide SSRs) microsatellites (56 and 21%, respectively) occurred in higher proportion than the trinucleotide (16.5%) and other types of microsatellite. The most likely explanation for this observation is that the majority of SSR loci integrated in the consensus map were derived from genomic DNA libraries that had been screened only for dinucleotide SSR probes (Ramsay et al. 2000; Li et al. 2003). The availability of different types of SSR loci in a given region (chromosome interval) will facilitate selection of the SSR repeat motifs of choice in a particular region of interest.

It is important to note that whenever possible, the primer sequences for the mapped loci were compiled and given in Table ESM S2. Availability of the primer sequences for a total of 580 SSR loci, approximately 75% of all loci integrated in the consensus map, at one place should accelerate the use of SSR markers in barley breeding activities. The primer sequences for 172 SSR loci (170 loci mapped in Varshney et al. 2006a and Marcel et al. 2007; two unpublished loci) have been made available in public domain for the first time. Primer sequences for the remaining 194 SSR loci can be obtained from Andreas Graner (for GBM loci) and Marion Röder (for GBMS loci), as per Material Transfer Agreement (MTA) basis. However, one marker (Bmac0029) is commercialised. The genotyping data made available for

all the 775 SSR loci (Table ESM S3) will allow the community to extend the dataset with their own dataset in future.

The majority of the SSR marker loci integrated on the consensus map have high information content. For instance, about 54% of the SSR loci for which the information was available have a PIC value >0.50. The compound and the dinucleotide microsatellite loci had higher PIC values than the trinucleotide and other types of SSR loci. This is probably due to the fact that only 12% of the compound and 37% of the dinucleotide SSR loci were derived from ESTs or genes (Ramsay et al. 2000; Li et al. 2003), while a much larger proportion of the trinucleotide (98.3%), tetranucleotide (90%), pentanucleotide (100%) and hexanucleotide (80%) SSR loci were derived from ESTs or genes (Thiel et al. 2003; Varshney et al. 2006a). Since ESTs or genes represent the transcribed regions of the genome (transcriptome), which are considered more conserved portions of the genome, transcriptome-derived markers generally have a lower polymorphism content (Varshney et al. 2005a). Nevertheless such markers are supposed to be more transferable between related species (Varshney et al. 2005b). Thus, depending on the objective, genomic DNA-derived SSR markers with higher PIC value (for breeding purpose) or EST/gene-derived SSR markers with a lower PIC value (for using across the cereal species) may be selected from the present consensus map. The highest correlation coefficient (r = 0.70) obtained with the 953 barley accessions through the world further demonstrates the robustness of the PIC values compiled for microsatellite loci on the consensus map.

Accuracy of the consensus SSR map

Although consensus maps represent the densest possible genetic maps, accuracy and quality of the developed consensus map is very important for its users. In order to construct an as accurate and precise consensus map as possible, a number of improved map construction programmes were used in the present study as compared to earlier studies (Karakousis et al. 2003; Somers et al. 2004; Qi et al. 2004). For instance, the recently developed computer program RECORD (Van Os et al. 2005a) was used for ordering the markers from the six individual linkage data sets and the linkage groups were sorted by graphical genotyping with help of Microsoft® Office Excel 2003. The programme RECORD employs a marker-ordering algorithm based on minimisation of the total number of recombination events in any given marker order (Van Os et al. 2005a). To be more accurate, the ordering of markers with RECORD programme was repeated three times for each individual linkage map. During the visual inspection of graphical genotypes, occurrence of singletons and other potential errors in the marker segregation data were identified. Because most singletons are scoring errors, these were replaced by missing values as suggested by Isidore et al. (2003) and Van Os et al. (2005b). The elimination of singletons solves most of the ordering ambiguities during the

mapping process, as the risk of cleaning data points that were not erroneous has a very limited effect on the marker ordering. The order of markers as given by RECORD is better than the order of markers as given by traditional linkage mapping software programmes like JoinMap® 3.0 and the simultaneous use of both programmes improves the construction of genetic linkage maps (Vromans et al. 2007).

A bridge marker is more reliable since it has a position on several populations. In case a mistake occurs in the map of one population, the error may be partly corrected by the position on the map of the other population. Therefore, the accurate identification of those bridge markers is of high importance and much attention was placed on assigning identical names to the bridge markers among the data sets. In the sets of marker segregation data obtained for different mapping populations, many inconsistencies especially in naming a particular SSR locus were found. Therefore, we suggested a slight modification in designation of SSR loci (Table ESM S1). We propose to use those designations of SSR loci in future studies in order to achieve a uniform convention.

Subsequently, with the corrected segregation data and with correct bridge markers, the final consensus map was calculated following the "neighbours" map approach described by Cone et al. (2002). In order to allow comparison of this map with other genetic maps, the barley BIN markers also have been integrated (Kleinhofs and Graner 2001; Marcel et al. 2007).

While utmost precautions were taken in preparing the consensus map, there could be some disagreement in the order of closely linked markers between the individual maps within some chromosome intervals. Such a disagreement may be due to the quality as well as the quantity and distribution along the chromosome of the bridge (common) markers used for preparing the consensus map, or to mapping populations, algorithm and stringency criteria of computer programmes. For example, the mapping populations for which the consensus map has been prepared have different numbers and different types of progeny lines. In smaller populations, the chance that informative recombinant progeny lines are present in the population to accurately position markers is lower than in larger populations. Also, the amount of recombination accumulated in RILs exceeds that in DH lines. Further, even for a given mapping population, different markers were mapped using different subsets of progeny lines in different laboratories. Therefore, the users of the consensus SSR map must consider that the marker order is conditioned by several factors like the progeny lines used and the position of crossovers along chromosomes within the progeny lines. The precise fine marker order may differ slightly in other populations and users may need to verify the order of closely linked markers in their mapping and breeding populations. However, we consider the order of the 496 bridge markers used to construct the framework of the consensus map to be highly reliable. The average distance between two consecutive bridge markers is equal to one marker per 2.1 cM, which shows the resolution of the map and the scale to which marker inversion

may occur. This resolution is less than half the size of the 5 cM sub-BINs. The sub-BINs are therefore a reliable reference for users of the consensus SSR map to select markers of interest.

About 10% of consecutive pairs of bridge markers are more than 5 cM apart, mostly in the distal parts of the linkage groups. Distances between pairs of consecutive bridge markers are much smaller around the centromeres because of suppressed recombination in the centromeric regions (Künzel et al. 2000). Differences in the order of markers between the SSR consensus map and previously published maps were therefore mostly observed around the centromeres.

Implications of the SSR consensus map

The present SSR consensus map has brought the majority of presently known barley SSR markers together to provide a good estimation of relative order and distance between them. The consensus map integrates already published (Saghai Maroof et al. 1994; Becker and Heun 1995; Liu et al. 1996; Struss and Plieske 1998; Ramsay et al. 2000; Li et al. 2003; Thiel et al. 2003) and very recently developed (mainly GBM and scssr; Rostoks et al. 2005; Varshney et al. 2006a; Marcel et al. 2007) barley SSR markers.

The primary use of the consensus map is in molecular mapping of traits and MAS in plant breeding. The precise marker order over short chromosome intervals (<5 cM) may not be that important to select progenies by marker-assisted approaches. Marker order of stretches of more than 5 cM, the size of a sub-BIN, is more relevant for that purpose. Here the consensus map provides a large number of markers along the length of each chromosome. This marker density allows a wide selection of markers that can be used to genotype individuals for detection of recombinants, fixation of loci to homozygosity, restoration of a recurrent genetic background or composition of complex genotypes combining several particular alleles (Varshney et al. 2004; Langridge and Chalmers 2004). Further, the information available on PIC value for a large number of markers will help users to select the most polymorphic markers from a region of interest on the genetic map. A putative function associated with genic-SSR loci makes them a useful resource for assaying functional variation in germplasm collections and natural or breeding populations (Varshney et al. 2005a). The integrated genic-SSR loci will not only be useful in barley genetics and breeding, but also for such activities in other cereals, as this class of SSRs are highly transferable among (closely) related species (Varshney et al. 2005a; 2005b).

The integrated SSR map could also help anchor the emerging physical map of barley (http://phymap.ucdavis.edu:8080/barley/). Those SSR markers with known genetic location could be used to screen BAC libraries allowing the positioning of BACs or BAC contig(s) onto the genetic map. Thus, the present consensus SSR map provides an opportunity to correlate genetic and physical maps (Varshney et al. 2006a).

In conclusion, we have brought together the vast majority of mapped barley microsatellite loci into a single consensus genetic map. The map provides molecular breeding strategies with a better choice of genetically located, high quality SSR markers, and, as a result, a higher probability of detecting polymorphic markers in any target chromosomal interval. In addition, it offers an opportunity to align established genetic and phenotypic maps with the emerging barley physical map and to initiate haplotype diversity and association studies with user friendly and informative molecular markers at a higher than previously possible resolution.

Note

Tables ESM are available with the online version of the article (DOI: 10.1007/s00122-007-0503-7). Details on all the possible features of the SSR markers integrated in the consensus map are given in Table ESM S1. The primer pairs for the SSR markers are given in Table ESM S2, while the genotyping data for all the SSR loci have been provided in Table ESM S3.

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CHAPTER 4

Dissection of the Barley 2L1.0 Region Carrying the '*Laevigatum*' Quantitative Resistance Gene to Leaf Rust Using Near Isogenic Lines (NILs) and Sub-NILs

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Submitted for publication

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Early aborted colony of P. hordei *without necrosis*

Dissection of the barley 2L1.0 region carrying the '*Laevigatum*' quantitative resistance gene to leaf rust using near isogenic lines (NILs) and sub-NILs

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Abstract Partial resistance to leaf rust (*Puccinia hordei* Otth) in barley is a quantitative resistance that is not based on hypersensitivity. This resistance hampers haustorium formation resulting in a long latency period in greenhouse tests. The three most consistent QTLs uncovered in the L94 × 'Vada' mapping population were introgressed by marker-assisted backcrossing into the susceptible L94 background to obtain near isogenic lines (NILs). We also developed the reciprocal Vada-NILs for the susceptibility alleles of those QTLs. The QTL *Rphq2* affected latency period of *P. hordei* more than the QTLs *Rphq3* and *Rphq4*. The NILs confirmed the contribution of *Rphq2* to partial resistance by prolonging the latency period by 28 hours on L94-*Rphq2* and shortening the latency period by 23 hours on Vada-*rphq2*. On the basis of flanking RFLP-based markers, *Rphq2* appeared to be located near the telomeric end of of the long arm of chromosome 2H, in a physical region of high recombination, making it the target QTL for map-based cloning. Microscopic observations on the NILs confirmed the non-hypersensitive nature of the resistance conferred by *Rphq2*. A high-resolution genetic map of the *Rphq2* region was constructed using a population of 39 sub-NILs with overlapping L94 introgressions in 'Vada' background across the region. *Rphq2* mapped about 1 cM proximal from the *MlLa* locus. By bulked segregant analysis and use of synteny with rice we developed additional markers, and fine-mapped *Rphq2* to a genetic interval of 0.11 cM that corresponds to a stretch of sequence of at most 70 kb in rice. Analysis of this rice sequence revealed predicted genes encoding two proteins with unknown function, retrotransposon proteins, peroxidase proteins and a protein similar to a MAP3K. Possible homologues of those peroxidases and MAP3K in barley are candidates for the gene that contributes to partial resistance to *P. hordei*.

Additionnal keywords: *Hordeum vulgare*; *Blumeria graminis* f.sp. *hordei*; quantitative trait locus (QTL); comparative mapping; substitution mapping

Introduction

Genetic dissection of a character of interest is an essential step towards the map-based cloning of the gene(s) underlying this character. Map-based cloning, also called positional cloning, is the process of identifying the genetic basis of a mutant phenotype by looking for linkage to markers whose physical location in the genome is known (Jander et al. 2002). Although map-based cloning is still considered as time-consuming and laborious, the availability of the whole genome sequences of *Arabidopsis* (Jander et al. 2002) and rice (Xu et al. 2005) greatly facilitates its process. For example, the occurrence of extensive synteny among cereal crops allows the use of the rice genomic sequence for comparative genome analysis (Devos 2005). Map-based cloning has been used to isolate a large number of genes that inherit according to Mendelian ratios but it has been considered problematic for quantitative trait loci (QTLs) since genotypes cannot be unambiguously recognised from phenotypes of individual plants (Remington et al. 2001). A QTL gene is characterised by its quantitative effect, is dependent on the genetic background and on its allelic form, and requires QTL-mapping software to establish its position (Niks et al. 2004). The development of QTL-near isogenic lines (NILs) allows the evaluation of a QTL in a nearly uniform genetic background, overcoming the difficulties of identifying QTL phenotypes. In a QTL-NIL, the target QTL becomes the major genetic source of variation because of the absence of other segregating QTLs. The QTL is considered Mendelised (Alonso-Blanco and Koornneef 2000). Then, by developing multiple sub-NILs with overlapping introgressions across the target region, substitution mapping can effectively dissect the QTL (Paterson et al. 1990). Indeed, most successes in cloning plant QTLs have been obtained by following this approach (Salvi and Tuberosa 2005).

At Wageningen University, the partial resistance of barley (*Hordeum vulgare* L.) to leaf rust (*Puccinia hordei* Otth) is studied since 1973. The measurement of latency period of the rust fungus has been shown to be the most reliable and effective method to quantify levels of partial resistance in a greenhouse test (Neervoort and Parlevliet 1978). A population of 103 F_9 recombinant inbred lines (RILs) was developed from a cross between L94 and 'Vada' (Qi et al. 1998a). L94 is a line from an Ethiopian landrace extremely susceptible to barley leaf rust. 'Vada' is an obsolete Dutch cultivar developed from the cross '*Hordeum laevigatum*' × 'Gold' (Dros 1957), which has a high level of partial resistance to *P. hordei* (Neervoort and Parlevliet 1978; Niks 1982). QTL mapping in L94 × 'Vada' identified six QTLs (Qi et al. 1998b). The three QTLs that showed the largest and most consistent effect (i.e. *Rphq2*, *Rphq3* and *Rphq4*) were introgressed into the susceptible L94 background by marker-assisted backcrossing to obtain NILs (Van Berloo et al. 2001). *Rphq2* is located on chromosome 2HL in the 2L1.0 region described by Dilbirligi et al. (2005). The 2L1.0 region is one of the largest gene rich regions in wheat and barley and is highly syntenic between those two cereals. The *Rphq2* locus, together with the powdery mildew (*Blumeria graminis* f.sp. *hordei*) resistance

gene *MlLa* (Giese et al. 1993) and the barley leaf stripe (*Pyrenophora graminea*) resistance gene *Rdg1a* (Thomsen et al. 1997; Arru et al. 2002), has been transferred to European barley cultivars from the 'botanical' barley line '*Hordeum laevigatum'* (Jensen and Jørgensen 1992). *Rphq3* is located near the centromere of chromosome 6H and *Rphq4* is on the satellite chromosome 5HS.

The objective of this study was to characterise macroscopically, microscopically and molecularly the three most consistent QTLs identified in order to select the best candidate for cloning a gene for partial resistance to barley leaf rust. Information on the size of the effect of each QTL in its NIL-background and on recombination frequencies in the three QTL regions are the basis on which to make this selection. The genetic region of *Rphq2* was saturated with molecular markers available in the literature, by following a bulk segregant analysis and by making use of the synteny between rice and barley. Finally, the gene for quantitative resistance to leaf rust *Rphq2* and the powdery mildew resistance gene *MlLa* were fine-mapped by substitution mapping.

Material and methods

Disease evaluations at the seedling stage

In all experiments, we used the long-time standard *P. hordei* isolate 1.2.1, which is a monospore culture derived from isolate 1.2 (Parlevliet 1976). The inoculum preparation and the inoculation were performed as described by Marcel et al. (2007). The latency period (LP) on each plant was evaluated by estimating the period (in hours) at which 50% of the ultimate number of pustules became visible. The relative latency period on seedlings (RLP50S) was calculated relative to the LP on L94 seedlings, where L94 was set at 100, as described by Parlevliet (1975).

We used the avirulent *B. graminis* f.sp. *hordei* isolate C15, kindly provided by Dr. M.S. Hovmøller (Danish Institute of Agricultural Sciences, Slagelse, Denmark), to map the resistance gene *MlLa* on our set of sub-NILs. The seeds were sown in trays of 37x39 cm, each of them containing two rows of 32 seeds. Four seeds were sown for each genotype. The susceptible barley cultivar 'Manchuria' and the near isogenic line Pallas-*MlLa* were included in each box. The first leaf of each seedling was fixed horizontally on the soil, adaxial side up, and trays were placed in a settling tower. Leaves infected with powdery mildew were collected from plants of 'Manchuria' and the spores were blown on two-weeks old seedlings by air compression within the settling tower. Each box received an average density of about 20 spores per mm^2. After seven days incubation in a greenhouse compartment where the temperature was set at 20°C day – 18°C night with 16 hours of light and a relative humidity of 60%, the infection types (IT) were scored according to the 0–4 scale proposed by Mains and

Dietz (1930). *MlLa* confers an intermediate reaction type characterised by an IT 3. The sub-NILs with an average IT < 3.5 were considered to carry the Vada-allele of *MlLa* while the sub-NILs with an average IT > 3.5 were considered to carry the L94-allele of *MlLa*.

Near isogenic lines development

NILs were previously developed through a marker-assisted backcross programme by incorporating the barley leaf rust resistance QTLs *Rphq2*, *Rphq3* and *Rphq4* (Qi et al. 1998b) into a L94 susceptible genetic background (Van Berloo et al. 2001). The obtained L94-*Rphq2* and L94-*Rphq3* NILs were crossed and, in the F_2 progenies, microsatellite markers were used to select a new NIL containing both QTLs in homozygous condition. To verify their quality, the L94-NILs were genotyped with an additional eleven amplified fragment length polymorphism (AFLP) primer combinations that resulted in 226 AFLP markers in L94 ×'Vada' (Marcel et al. 2007).

The same procedure was applied to develop the reciprocal NILs containing the L94 allele of *Rphq2*, *Rphq3*, *Rphq4* and *Rphq6* in the genetic background of 'Vada'. Twenty AFLP primer combinations were selected to screen the BC_3 generation of the Vada-NILs. These primer combinations resulted in 157 amplified fragments that occurred in L94 but not in 'Vada'. Only L94 amplified fragments were informative, since all plants in the backcross population carried at least one allele from the recurrent parent, 'Vada'. For the next backcross generations, we used only the primer combinations amplifying AFLP markers which detected L94-derived fragments in the previous generation. Additionally, 10 microsatellites and 9 locus specific PCR markers were used to cover areas of the genetic linkage map uncovered by the AFLP markers or to monitor the introgressions carrying the target QTLs. In the BC_5 generation, microsatellite markers were used to select a line with the L94 alleles of *Rphq2* and *Rphq3*. The selected BC_5 line was selfed to obtain a new NIL containing both QTLs in homozygous condition.

The RLP50S on the L94-NILs and Vada-NILs was evaluated in six experiments. Each experiment contained five seedlings per genotype.

Microscopic characterisation of the L94-NILs

The reaction of L94, 'Vada' and NILs were characterised by fluorescence microscopy. The experiment was conducted by inoculating ten seedlings of each genotype, sown in a single tray, with the *P. hordei* isolate 1.2.1. Middle segments of 3-4 cm^2 from the first leaves of two seedlings per genotype were collected at 110 hours post-inoculation (hpi). The eight remaining seedlings were used to measure the LP on each genotype. This experiment was performed two times. The collected segments were processed for fluorescence microscopy

(Rohringer et al. 1977) but instead of Calcofluor we used Uvitex 2B for staining (Ciba-Geigy). The preparations were examined at 100x – 400x magnification with an epifluorescence microscope Axiophot I (Zeiss, Germany). From 62 to 132 (average 98) infection units per leaf segment were scored and classified according to their stage of development (Niks 1982). Infection units that had not formed any haustorial mother cell were ignored. Infection units that formed a primary infection hypha and no more than six haustorial mother cells were considered as early aborted. Infection units with more than six haustorial mother cells were classified as established. Necrotic host cells displayed a golden yellow autofluorescence. The number of infection units associated with host cell necrosis was recorded. We measured the longest diameter of the established colonies by eye-piece micrometer.

Conversion of RFLP markers to locus specific PCR markers

Twenty-three primer pairs were designed on barley restriction fragment length polymorphism (RFLP) sequences to develop markers flanking *Rphq2*, *Rphq3* and *Rphq4*. The DNA sequences were downloaded from the GrainGenes database (http://wheat.pw.usda.gov/GG2/index.shtml). The optimal annealing temperature of each primer pair was determined by gradient-PCR. The generated PCR products of parental lines L94 and 'Vada' were then digested with 24 restriction enzymes (CAPS-kit: Bai et al. 2004) in order to detect polymorphism. If after testing those 24 restriction enzymes no polymorphism was detected, PCR products were sent for direct sequencing (BaseClear, Leiden, the Netherlands). Primer design and sequence analysis were done with the Lasergene software (DNASTAR® 6.1 Inc., Madison, WI, USA). The DNA extraction and PCR procedure was done as described in Marcel and Niks (2004). Detailed information on the locus specific PCR markers used in this study can be obtained from Marcel et al. (2007).

The sequences of two additional primer pairs were obtained from Hori et al. (2005) for k00345 and from Mohler and Jahoor (1996) for MWG097.

Marker assisted selection with microsatellite markers

We searched within the mapped barley microsatellites (Varshney et al. 2007) to find additional co-dominant markers within the *Rphq2*, *Rphq3* and *Rphq4* regions. The microsatellites used for the marker assisted selection (MAS) of *Rphq2* were GBM1012, GBM1475, GBMS216 and GBMS128b and for the MAS of *Rphq3* were Bmac0018, Bmag0009, HVM14, HVM22a, HVM65, HVM74, GBM1063 and GBM1076. No microsatellite was found within the *Rphq4* introgression. PCR conditions were according to Varshney et al. (2007). The primers were synthesised and the reverse primers IRD-labelled at

Biolegio BV (Nijmegen, The Netherlands). The PCR product was loaded on a 5.5% denaturing polyacrylamide gel (5.5% Ready to use Gel Matrix, KB Plus, Westburg) and visualised on a LI-COR 4200 DNA automated sequencer (LI-COR® Biosciences, Lincoln, NE).

Bulked segregant analysis with AFLP

A bulked segregant analysis (BSA) was performed with AFLP primer combinations on the parents, L94 and 'Vada', on the NIL L94-*Rphq2* and on a susceptible and a resistant pool of RILs derived from L94 × 'Vada'. The pools were composed of equal volumes of AFLP pre-amplification products of seven RILs having the L94-alleles for the markers flanking *Rphq2* and of eight RILs having the Vada-alleles for the same flanking markers, for the susceptible and the resistant pools, respectively. The AFLP fingerprints were generated as described by Marcel et al. (2007). The analysis was conducted exclusively with *Pst*I/*Mse*I restricted DNA.

AFLP markers were converted to locus specific PCR markers according to the method of Brugmans et al. (2003).

Comparative mapping in rice and barley

We performed targeted synteny-based marker saturation for the *Rphq2* locus following the procedure proposed by Perovic et al. (2004). The blastn function of the KOME database (http://cdna01.dna.affrc.go.jp/; Kikuchi et al. 2003) was used for the homology search between barley expressed sequence tags (EST) and rice clones. The barley ESTs used for this search mapped distally to the microsatellite GBM1475 on the transcript map of barley (Stein et al. 2007). The predicted rice coding sequences of the identified bacterial artificial chromosome (BAC) clones were used for blastn analysis in the barley TIGR Gene Indices database (http://www.tigr.org/tdb/tgi/index.shtml). Annotation and gene prediction of rice BAC sequences was obtained from the TIGR Rice Genome Browser web page (http://www.tigr.org/tigr-scripts/osa1_web/gbrowse/rice). The barley EST with the highest homology to each predicted rice gene, above a threshold E value $\leq 10^{-15}$, was selected as candidate probe for genetic mapping.

Substitution mapping with sub-NILs

Two BC_4S_1 plants were identified that were heterozygous for the DNA introgressions spanning the *Rphq2* region. Their BC_4S_2 progenies were genotyped with the microsatellites GBM1475 and GBMS216 to select Vada-*rphq2* and Vada-*rphq2*^{b}, and to identify plants that recombined within the introgression. Ten BC_4S_3 seedlings per recombinant line were tested

with the flanking microsatellites to identify plants in the progenies that were homozygous for the recombined marker allele. Those plants were selfed to obtain sub-NILs. Sub-NILs are therefore homozygous lines that have recombined within the segment introgressed in the corresponding NIL. All sub-NILs were genotyped with all molecular markers located distally to GBM1475 to construct a high resolution genetic map of the *Rphq2* region. The sub-NILs were subjected to disease tests. The LP of *P. hordei* 1.2.1 was estimated in two experiments on five seedlings per sub-NIL. The IT against *B. graminis* C15 was estimated on four seedlings per sub-NIL.

Total DNA was extracted from leaves of the BC_4S_2 and BC_4S_3 plants following a method based on alkaline (NaOH) solution described by Wang et al. (1993), adjusted for 96-well format. This simple method allowed extracting DNA from a large number of plants in a limited amount of time, which suits to the screening of large populations needed to identify rare recombinants.

Statistical analyses

Linkage analyses were done with JoinMap® 3.0 (Van Ooijen and Voorrips 2001) applying the Kosambi's mapping function. QTL-mapping was performed using MapQTL® 5.0 (Van Ooijen 2004). Interval Mapping (IM) was run and markers at the LOD peaks were taken as cofactors for running the restricted-MQM mapping method (rMQM). The proportion of explained phenotypic variance was estimated with the rMQM results.

The analyses of variance with RLP on NILs and sub-NILs, and percentage of early abortion and colony length on NILs were performed with GenStat® Release 8.1 (2005). A Duncan's multiple range test ($P < 0.05$) was used to compare all pairs of means between NILs. The $LSD_{0.05}$ (least significant difference, $P < 0.05$) was used to declare the mean of a sub-NIL significantly different or not from the mean of 'Vada'.

Results

Construction of QTL-NILs containing L94 and 'Vada' introgressions

Two sets of NILs were generated in the genetic backgrounds of L94 and 'Vada' (Fig. 1). Van Berloo et al. (2001) developed NILs containing the QTLs *Rphq2*, *Rphq3* and *Rphq4* in the susceptible background of L94. Genotyping with 226 new AFLP markers resulted in an average distance between adjacent markers of 4.1.cM and revealed one unwanted 'Vada' introgression at the telomeric end of chromosome 3H in L94-*Rphq3* and two unwanted 'Vada' introgressions on chromosomes 3H and 6H in L94-*Rphq4*. L94-*Rphq4* was backcrossed a fourth

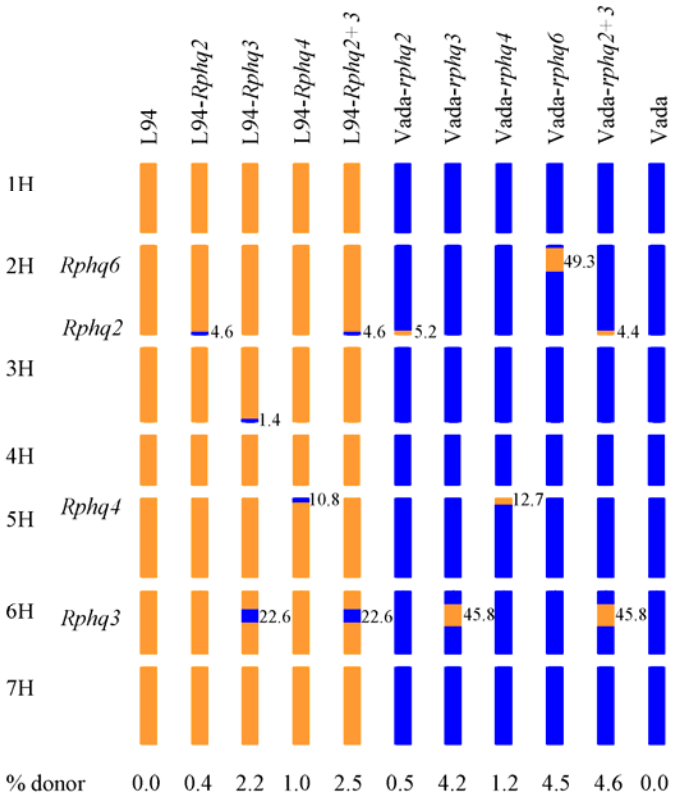


Figure 1. Graphical genotypes of 4 NILs carrying the Vada-allele of QTLs or combination of QTLs in the susceptible L94 genetic background and of 5 reciprocal NILs carrying the L94-allele of QTLs or combination of QTLs in the partially resistant Vada genetic background. The name of a QTL is indicated at its approximate position on the corresponding linkage group. The size of the introgressions (in cM) are indicated on the right side of the chromosome bars. % donor is the estimated percentage of donor-derived genome remaining in the corresponding NIL.

time to the recurrent parent L94 in order to select against the two unwanted 'Vada' introgressions. A NIL combining the two QTLs *Rphq2* and *Rphq3* was successfully selected with microsatellite markers in a segregating F_2 progeny derived from a cross between L94-*Rphq2* and L94-*Rphq3* (Fig. 1).

Reciprocal Vada-NILs were generated by selecting for a single donor genome introgression carrying *Rphq2* or *Rphq6* at the BC_4 generation and *Rphq3* or *Rphq4* at the BC_5 generation. The 157 AFLP markers used to select the Vada-NILs did not reveal any unwanted L94 fragment. The average distance between two adjacent AFLP markers was 5.6 cM. Plants containing interesting recombination events for fine-mapping the QTLs *Rphq2* and *Rphq3* were identified and retained during the selection process (i.e. Vada-*rphq2*^{b}, Vada-*rphq2*^{c} and Vada-*rphq3*^{b}). As counterpart to the L94-NILs, also a Vada-NIL combining the QTLs *Rphq2* and *Rphq3* was selected in the BC_5 generation. All BC_4 and BC_5 selected plants were selfed to select for NILs with homozygous introgressions.

On seedlings, *Rphq2* prolongs the latency period of *P. hordei* more than *Rphq3*

The effect of a QTL may depend on its genetic background and different QTLs may have different size of effect. Therefore, it is necessary to evaluate the phenotypes of the NILs before embarking on map-based cloning experiments. Greenhouse disease test on seedlings is a fast and reliable method for the phenotypic evaluation of the QTL-NILs in repeated

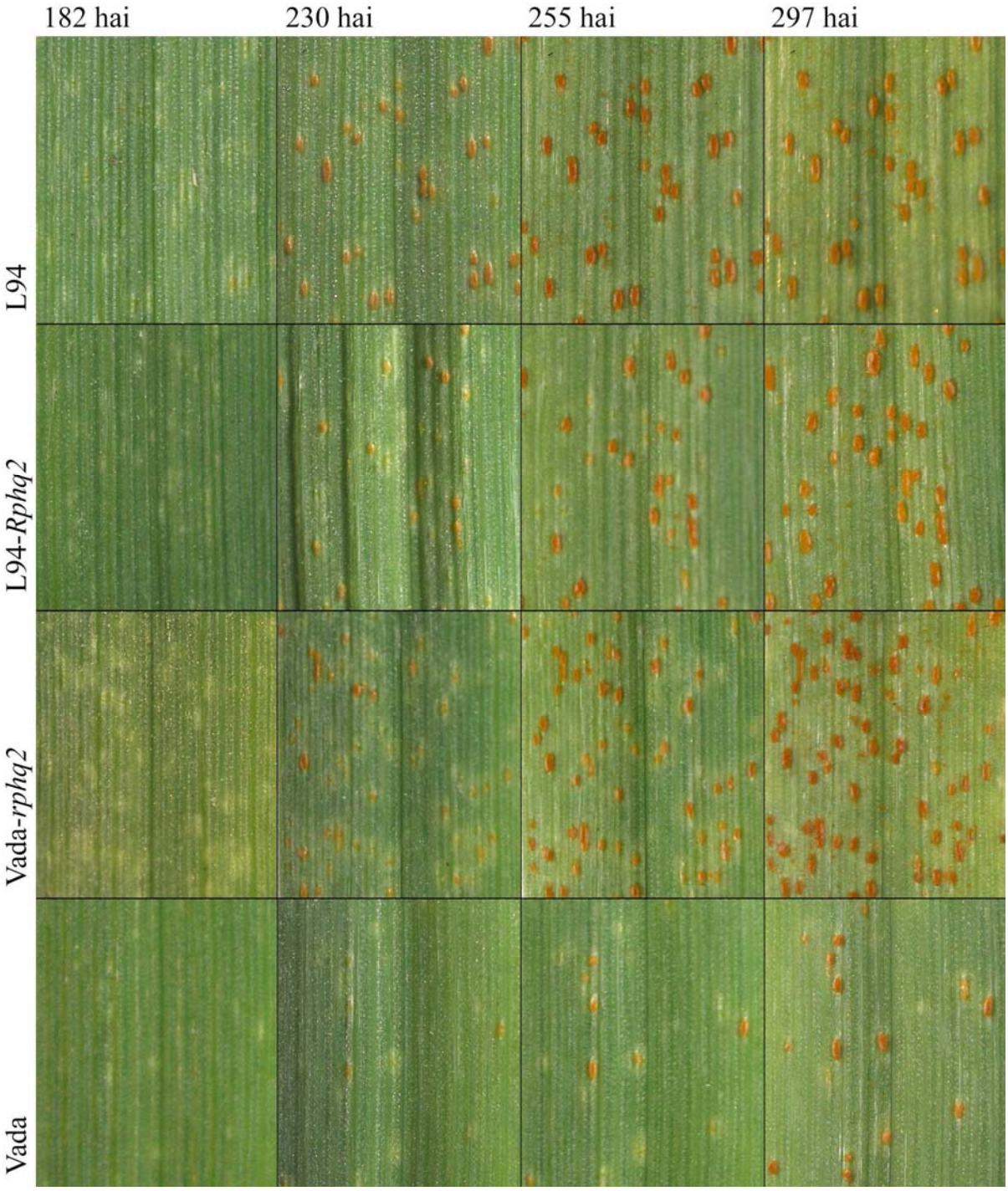


Figure 2. First leaf of barley seedlings from the susceptible line L94, the NILs L94-*Rphq2* and Vada-*rphq2* and the partially resistant line 'Vada' at 182, 230, 255 and 297 hours after inoculation (hai) with *P. hordei* 1.2.1. For each line, the same leaf area is shown at four different time points. The latency period on the corresponding seedlings were 190, 208, 205 and 237 hours, respectively.

experiments. In the RIL population, Qi et al. (1998b) identified *Rphq2* and *Rphq3* as being the two QTLs with the greatest effect in the seedling stage, while they identified *Rphq4* and *Rphq6* only in the adult plant stage and in the field. The evaluation of the NILs confirmed that

Rphq2 had a significant effect in seedlings (Table 1; Fig. 2). *Rphq2* prolonged the LP by 28 hours on L94-*Rphq2* while the alternative *rphq2*-allele shortened the LP by 23 hours on Vada-*rphq2*. In the NILs, *Rphq3* did not have a significant effect in seedlings compared to the parental lines L94 and 'Vada' (Table 1). Nevertheless, *Rphq3* prolonged the LP by 9 hours on L94-*Rphq3* while the alternative allele shortened the LP by 7 hours on Vada-*rphq3*. The combination of *Rphq2* and *Rphq3* in the same NILs background resulted in levels of resistance higher than the ones of any NIL with a single QTL introgression (Table 1). However, these differences were not always statistically different. In L94 and in 'Vada' background, both introgressed *Rphq2* and *Rphq3* alleles, or *rphq2* and *rphq3* alleles, even resulted in a relative latency period (RLP) similar to that on 'Vada' and L94, respectively. It indicates that these two QTLs explain nearly all the genotypic variation observed at seedling stage. As expected on the basis of the results of Qi et al. (1998b), the LP of the rust was on seedlings of L94-*Rphq4*, Vada-*rphq4* and Vada-*rphq6* not significantly different than on L94 and Vada, respectively (Table 1).

Table 1. Relative latency period at seedling stage of *P. hordei* isolate 1.2.1 on barley RILs and NILs that differ for *Rphq*-genes

	Recombinant Inbred Lines[1]			Near Isogenic Lines	
	Fitted value[2]	Mean[3]	s.e.[4]	Mean[5]	s.e.
L94-*Rphq2*	110	109^{b}	2.17	116^{bc}	1.80
L94-*Rphq3*	108	109^{b}	1.10	106^{ab}	1.80
L94-*Rphq4*	102	103^{ab}	1.93	100^{a}	2.07
L94-*Rphq2+3*	117	119^{c}	0.91	120^{bc}	1.77
L94	**-**	**101^{a}**	**1.44**	**100^{a}**	**1.77**
Vada-*rphq2*	113	110^{b}	1.23	115^{abc}	1.83
Vada-*rphq3*	115	109^{b}	1.68	125^{c}	2.12
Vada-*rphq4*	121	124^{c}	1.93	121^{c}	1.87
Vada-*rphq6*	122	124^{c}	1.88	128^{c}	2.19
Vada-*rphq2+3*	106	106^{ab}	1.83	106^{ab}	2.54
Vada	**-**	**124^{c}**	**1.51**	**127^{c}**	**1.80**

[1] According to Qi et al. (1998b)

[2] Theoretical value calculated based on the RIL-population mean and the allelic effect of each QTL as determined by MapQTL® 5.0

[3] Mean value of the RILs carrying the QTL(s) allele considered and the QTL(s) allele of the recurrent parent at the other QTLs predicted from regression model; means followed by a common letter are not significantly different according to Duncan's test ($P < 0.05$)

[4] Standard error estimates

[5] Mean value of the considered NIL predicted from regression model; means followed by a common letter are not significantly different according to Duncan's test ($P < 0.05$)

Microscopic observations confirm the pre-haustorial type of resistance conferred by *Rphq2*

The ranking of the genotypes on the basis of microscopically assessed percentages of early aborted colonies and sizes of established colonies were similar to the ranking on the basis of the macroscopically assessed levels of partial resistance (Fig. 3). 'Vada' and Vada-*rphq3* caused the highest RLP (Fig. 3a), and had a high percentage of early aborted colonies (Fig. 3b) and a short diameter of established colonies (Fig. 3c). A high degree of early abortion without plant cell necrosis is indicative of the failure of haustorium formation by the infection unit, which results in low levels of infectibility by *P. hordei* as observed on 'Vada' (Niks 1982, 1983a, 1986). L94-*Rphq2* and L94-*Rphq2+3* had significant macroscopic and microscopic effects on the level of partial resistance (Fig. 3). The percentage of early aborted colonies in L94-*Rphq2* was not significantly different from the one in L94 in this experiment

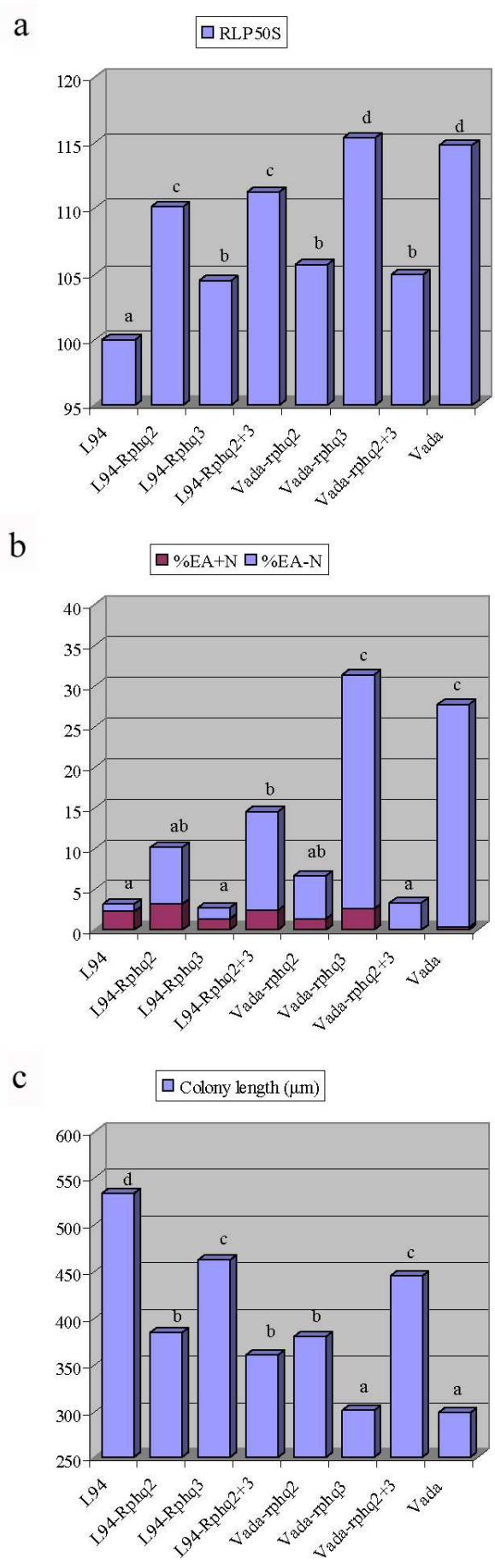


Figure 3. Histograms of the relative latency period at seedling stage, or RLP50S, (**a**); the proportion of early aborted colonies associated (%EA+N) or not (%EA-N) with host cell necrosis (**b**); and the length of established colonies in micrometers (µm) (**c**) after infection with *P. hordei* isolate 1.2.1 on L94, 'Vada' and on QTL-NILs. For microscopic observations, leaf segments were sampled 110 hours after inoculation. Similar letters on bars indicate that the means do not differ significantly according to Duncan's test ($P < 0.05$).

but was in quantity three times higher than in L94, L94-*Rphq3* or Vada-*rphq2+3* (Fig. 3b). About 69% of the early aborted colonies in L94-*Rphq2* were not associated with host cell necrosis, confirming the predominantly pre-haustorial type of resistance conferred by this gene. The total proportion of colonies (early aborted and established) associated with host cell necrosis ranged from 16% for Vada-*rphq2* to 53% for Vada-*rphq2+3*. However, the necrosis occurred frequently as only one cell in relatively large established colonies, and was not particularly strong in 'Vada' or NILs carrying *Rphq2* and *Rphq3*. This suggests that the necrosis was not a relevant factor explaining the level of partial resistance conferred by the QTLs. The four lines carrying the L94 allele of *Rphq2* had consistently a lower RLP (Fig. 3a), a lower percentage of early aborted colonies (Fig. 3b) and a longer diameter of established colonies (Fig. 3c) than the four lines carrying the Vada-allele of *Rphq2*.

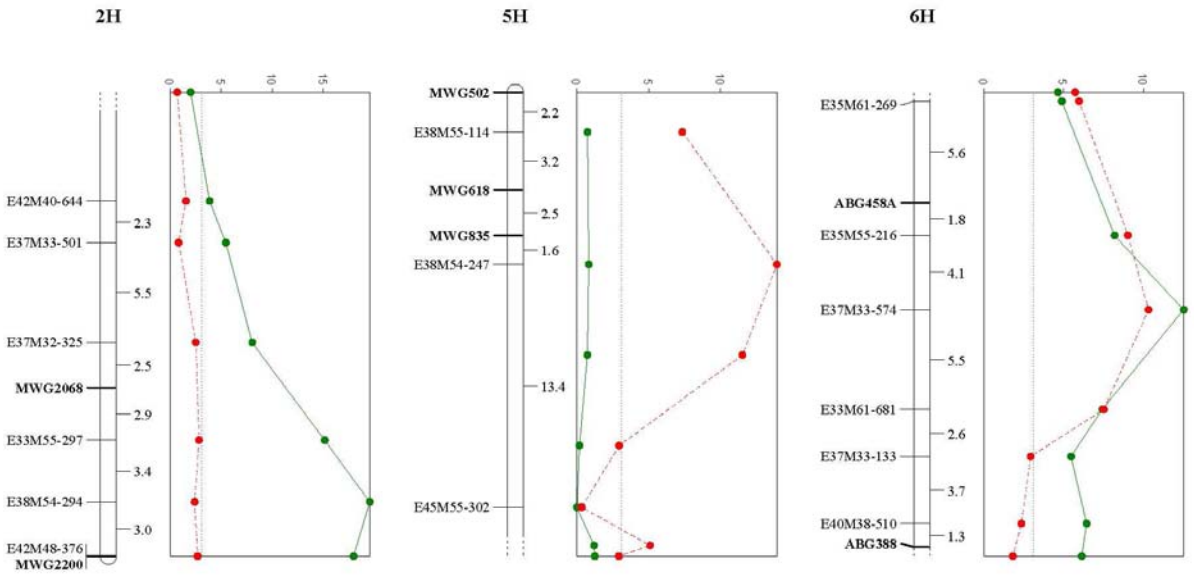


Figure 4. LOD profiles of *Rphq-2*, *Rphq-3* and *Rphq-4* along the genetic distances of the L94 × 'Vada' genetic linkage map, at the seedling stage (*green solid line*) and at the adult plant stage (*red dash line*), obtained by restricted-MQM mapping with the data of Qi et al. (1998b). Marker names are on the left side of the chromosomes and the genetic distances between two consecutive loci (in cM) are indicated on the right side of the chromosomes. Markers in *bold* are locus specific PCR markers converted from RFLP markers mapped on the physical maps of Künzel et al. (2000).

Selection of *Rphq2* as the target QTL to clone a gene for partial resistance

Nine of the twenty-three primer pairs designed on barley RFLP sequences were converted into locus specific PCR markers and mapped in the L94 × 'Vada' population (Marcel and Niks 2004; Marcel et al. 2007). Three of the mapped primer pairs gave direct sequence characterised amplified region (SCAR) markers (13%), ABG458A, MWG618 and MWG835, while the 6 others gave cleaved amplified polymorphic sequence (CAPS) markers (26%),

Prx2_*Aci*I, MWG2200_*Ava*I, MWG2068_*Hae*III, cMWG679_*BssK*I, ABG388_*Nla*III and MWG502_*Hpa*II. With the exception of cMWG679, all markers mapped in the vicinity of the target QTL. The CAPS marker cMWG679 mapped at the telomeric end of chromosome 6HS in L94 × 'Vada' (data not shown) while the corresponding RFLP marker had been mapped 60 cM proximal on that same chromosome arm (Graner et al. 1991). MWG2200 and MWG2068 flanked *Rphq2* on chromosome 2HL (Fig. 4) and delimit a physical region of high recombina-

Table 2. Genetic distance between the converted RFLP markers flanking the three QTLs and estimation of the physical to genetic ratio within the marker intervals based on previous studies

QTL	Marker interval	Genetic distance (cM)	Physical to genetic ratio (Mb/cM)	
			Künzel et al. 2000	Stephens et al. 2004
Rphq2	MWG2068-MWG2200	7.4	~ 1.1	1.8
Rphq3	ABG458A-ABG388	19.2	≤ 42.0	28.8
Rphq4	MWG502-MWG835	9.2	≤ 0.3	0.9

Table 3. Locus specific PCR markers developed from barley ESTs homologous to predicted genes from rice chromosome 4

Name	Type	Chr.	Restriction enzyme(s)	Tm (°C)[1]	Primer sequences (5' – 3')
WBE001	SSR	2H	-	56°	F: acgcacccgcccctgtttatct
					R: gctgccgtcgaggagggtgttc
WBE110[2]	CAPS	2H	*Hae*III; *Aci*I	60°	F: gcaggaagcgaaggtggcaatagc
					R: ccgaacagggaaacaccgacgaac
WBE111[2]	SCAR	2H	-	60°	F: ggggctcatccgcatcttctt
					R: tcagcaatcacggcaactaaacaa
WBE112[2]	CAPS	3H	*Mwo*I	61°	F: ctcgccccctcgcctacttcctc
					R: atctccgggtcctgctggctcctc
WBE113[2]	CAPS	2H	*Mbo*I	61°	F: tcctcgccctcttcatcatcctca
					R: gtagctgccctcccctcgttcac
WBE114	CAPS	2H	*Hpa*II; *Msp*I	58°	F: ggcgacctccagcgtatc
					R: gtggttcggtccttgatgag
WBE115	SCAR	2H	-	61°	F: ggcggtcggcatcgtccagt
					R: atgcgtccacaaaaccaatcttca
WBE116	dCAPS	2H	*Mse*I	61°	F: gggccggtcaccacgctctac
					R: ctacctccacttcaatcggcgatta

[1] Optimum annealing temperature determined by gradient PCR

[2] Data obtained from Marcel et al. (2007)

tion of 1.1 to 1.8 Mega-bases per centiMorgan (Mb/cM) (Table 2). ABG458A and ABG388 flanked *Rphq3* and mapped on both sides of the centromeric region of chromosome 6H (Fig. 4). They delimit a physical region of suppressed recombination of 28.8 to 42.0 Mb/cM (Table 2). MWG502 and MWG835 are closely linked to *Rphq4* on chromosome 5HS (Fig. 4) and delimit a physical region of very high recombination with less than 0.9 Mb/cM (Table 2).

Rphq2 is the easiest QTL to detect in seedlings of the corresponding L94-NIL and Vada-NIL (Table 1; Fig. 2). Microscopic observations confirmed the pre-haustorial type of resistance conferred by *Rphq2* (Fig. 3). On the basis of flanking RFLP-based markers, *Rphq2* appeared to be also located in a physical region of high recombination (Table 2), making it the QTL of choice for map-based cloning.

Marker saturation of the region containing *Rphq2* by BSA and synteny-based approaches

To efficiently identify molecular markers linked to *Rphq2*, on the distal end of chromosome 2HL, a BSA was performed by using AFLP on resistant and susceptible pools of L94 × 'Vada' RILs and on the NIL L94-*Rphq2*. By using a total of 48 *Pst*I/*Mse*I primer combinations, 8 AFLP markers were identified that were present only in the resistant pool, in 'Vada' and in L94-*Rphq2*, and 5 AFLP markers were identified that were present only in the susceptible pool and in L94. All 13 AFLP markers mapped in the L94 × 'Vada' RIL population within the 4.6 cM 'Vada' genomic segment introgressed into L94-*Rphq2*. Two previously mapped *Eco*RI/*Mse*I AFLPs, E40M32-402 and E42M48-376, and one new *Pst*I/*Mse*I AFLP, P15M53-435, were successfully converted into one CAPS and two SCAR markers designated caE40M32-402, scE42M48-376 and scP15M53-435. In L94 × 'Vada', caE40M32-402, scE42M48-376 and scP15M53-435 mapped at the expected position of the corresponding AFLP markers.

We further saturated the region of *Rphq2* with molecular markers by exploiting the synteny between rice and barley. The alignment of the genomes of major grass species indicated a syntenic relationship between the *triticeae* chromosome 2 and the rice chromosomes 4 and 7 (Moore et al. 1995; Devos 2005). The blastn analysis of barley ESTs identified a region on rice chromosome 4 as being syntenic with the region of *Rphq2*. The rice target region was covered by a contig of four BAC clones spanning a physical distance of 562 kb: OSJNBa0088H09, OSJNBa0070M12, OSJNBa0039K24 and OSJNBb00220J19 (Fig. 5c). We designed 19 primer pairs based on the sequences of 19 barley ESTs with highest homology to 19 predicted rice genes (3.10^{-19} < Expect < 3.10^{-238}) from the four rice BACs. Eight of the 19 primer pairs designed were converted into locus specific PCR markers and mapped in the L94 × 'Vada' population (Table 3). Seven markers (WBE001, WBE110, WBE111 and WBE113 to -116) mapped on barley chromosome 2HS within the L94 genomic segment introgressed in Vada-*rphq2* (Fig. 5b,c), while the eighth (WBE112: Expect = 3.10^{-51})

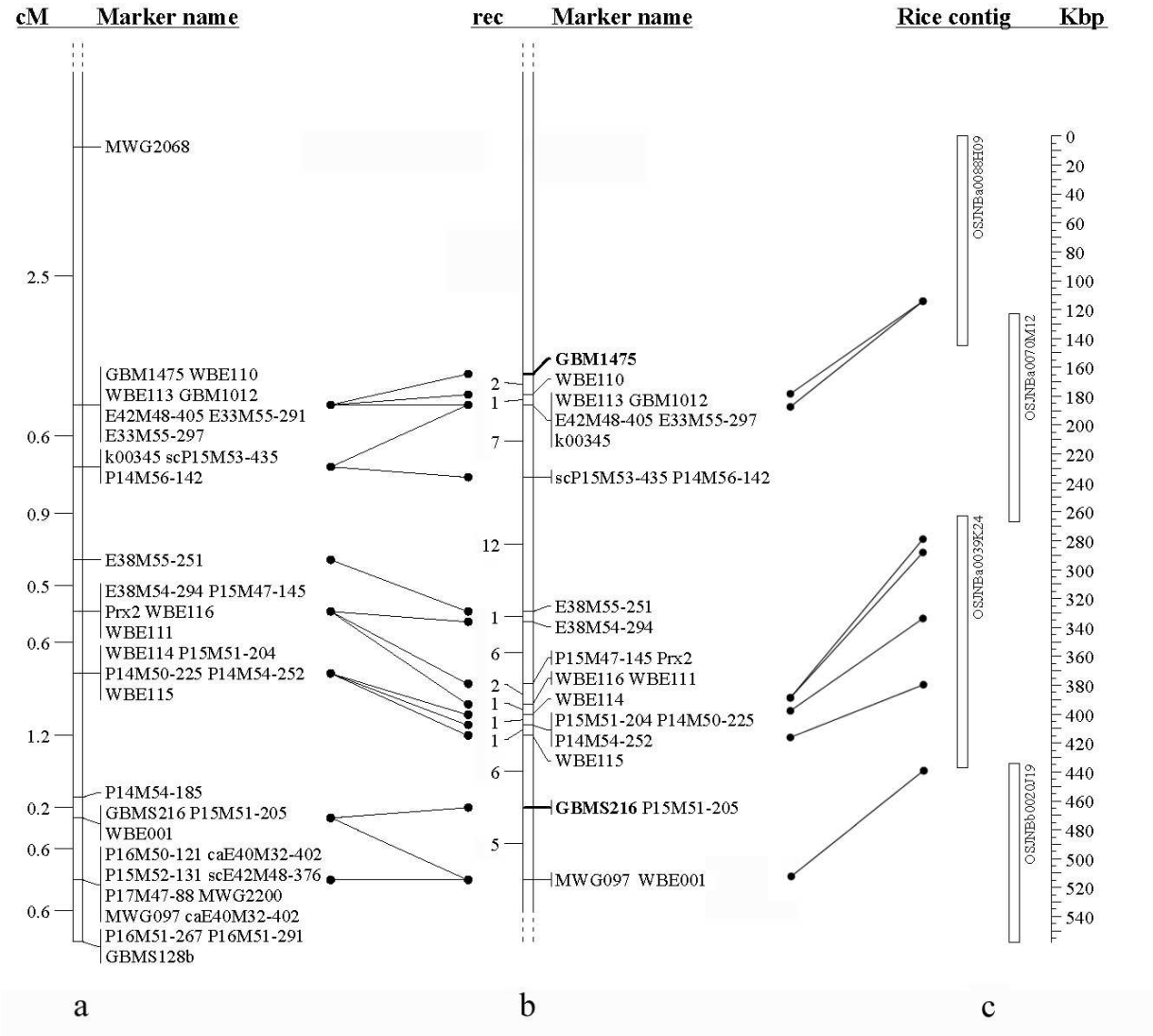


Figure 5. Alignment of two genetic linkage maps covering the *Rphq2* region on barley chromosome 2HL with the homologous region on rice chromosome 4. The first map (**a**) was constructed using a population of 103 RILs derived from the cross L94×'Vada'. Microsatellites GBM1475 and GBMS216 (in *bold*) were used to screen a population of 923 plants, and the resulting 39 sub-NILs used to construct the second map (**b**). The seven markers derived from rice ESTs (WBE001, WBE110, WBE111, WBE113, WBE114, WBE115 and WBE116) allowed alignment to a rice physical contig of 557.5 kb comprising four BAC clones (**c**). *Arrows* between (**b**) and (**c**) indicate the position of homologues present in the rice sequence. Genetic distances (cM) observed for specific intervals are shown in (**a**) while the number of recombination identified between consecutive markers are shown in (**b**). Physical scale (Kbp) in rice is indicated on the right in (**c**).

mapped on chromosome 3HS. Those EST-based markers were designated WBE*nr* for Wageningen Barley EST. WBE110 was the best barley homologue to two predicted rice genes on OSJNBa0088H09 that encode exocyst subunit Exo70 proteins and WBE114 was the best barley homologue to three predicted rice genes on OSJNBa0039K24 that encode peroxidase proteins. Except for an inversion between WBE110 and WBE113, the order of the

WBE*nr* markers on the barley high-resolution map (Fig. 5b) was in agreement with the order of the predicted genes on rice chromosome 4.

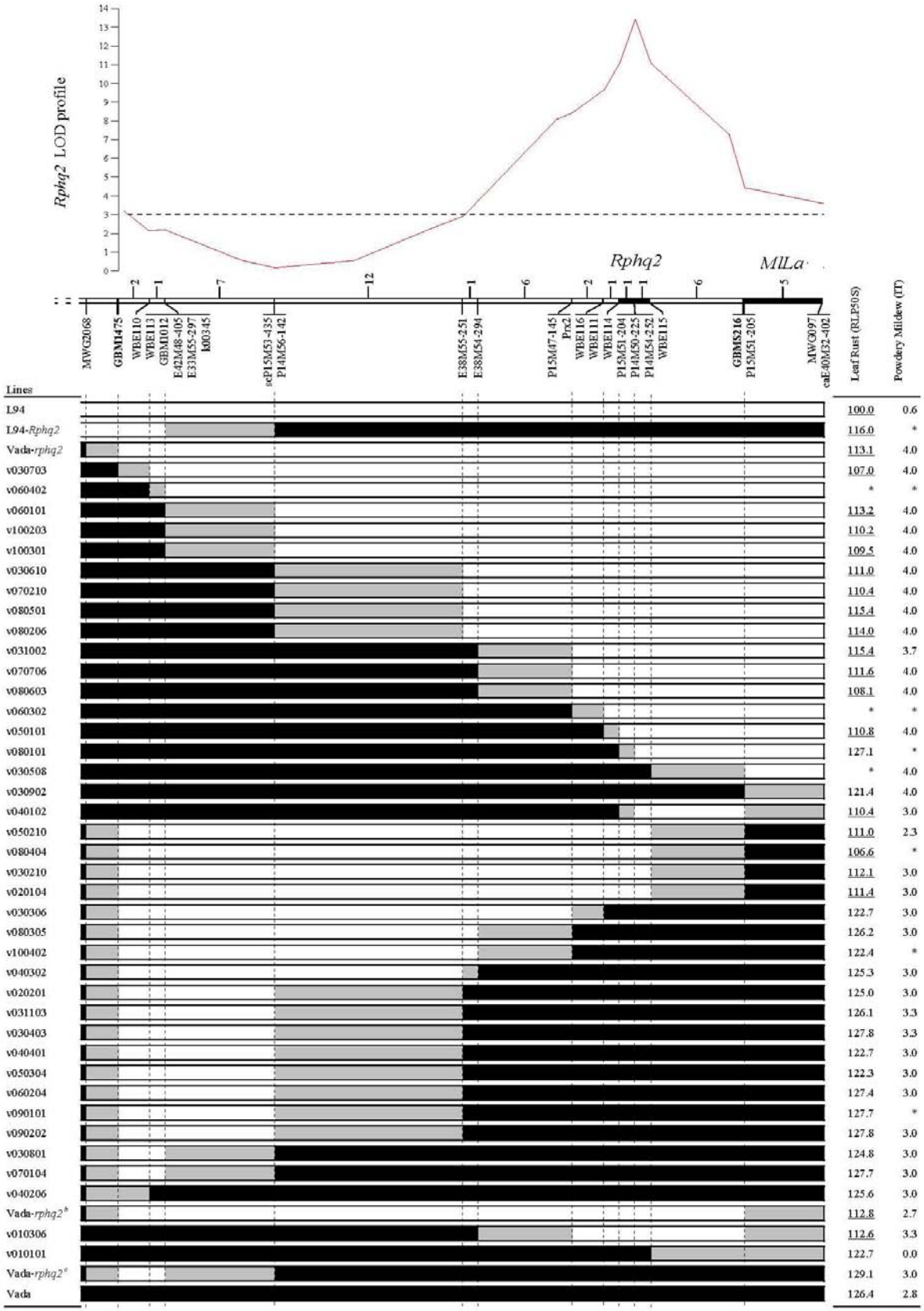
Altogether, the employed strategies saturated the region of *Rphq2* with an average density of one marker per 0.14 cM. Thirty-six markers mapped within the 5.2 cM L94 genomic segment introgressed in Vada-*rphq2* (Fig. 6): 8 *Eco*RI/*Mse*I AFLPs identified by Qi et al. (1998a), 13 *Pst*I/*Mse*I AFLPs identified by BSA (this study), 2 RFLPs converted into locus specific PCR markers (this study), 7 EST-based markers developed by synteny with rice (this study), MWG097 obtained from Mohler and Jahoor (1996), k00345 obtained from Hori et al. (2005) and 4 SSRs obtained from Varshney et al. (2007). Only the 29 most distal markers mapped within the 'Vada' genomic segment introgressed in L94-*Rphq2* (Fig. 6).

Substitution mapping places *Rphq2* to a genetic interval of 0.11 cM

The genetic fine-mapping of a target locus is an essential step preceding the map-based isolation of a gene. We screened 923 BC_4S_2 seedlings (i.e. 1,846 gametes) segregating for the L94 fragments introgressed in Vada-*rphq2* or in Vada-*rphq2*^{b} with the microsatellites GBM1475 and GBMS216. A set of 39 seedlings was identified that recombined between these two markers, implying a 2.1% recombination rate (Fig. 5b). This is two times less than the original 4.6 cM genetic interval calculated between those markers in the L94 × 'Vada' map (Fig. 5a). The disease test on sub-NILs resolved *Rphq2* in a single, incompletely dominant gene co-segregating with the AFLP markers P15M51-204, P14M50-225 and P14M54-252 (Fig. 6). Sub-NILs with an RLP value statistically significantly lower than the RLP on 'Vada' were considered to carry the L94 allele of *Rphq2*, while sub-NILs with a RLP value not statistically different from the RLP on 'Vada' were considered to carry the Vada-allele of *Rphq2*. The two resulting groups of sub-NILs, distinguished according to their phenotype, did not show any discrepancy with the corresponding genotypes (Fig. 6). The minimum difference between RLP50S values of two sub-NILs carrying different alleles of *Rphq2* was equal to 6 (i.e. v031002 and v030902) corresponding to a prolongation of the latency period of the leaf rust fungus of approximately 11 hours, while the average difference

Figure 6. (Next page). Graphical genotypes, leaf rust (latency period, RLP50S) and powdery mildew (infection type, IT) phenotype means, and RLP50S LOD profile for NILs and sub-NILs covering the L94 introgression on Vada-*rphq2* NILs. *Open bars* represent homozygous L94, *solid bars* represent homozygous Vada and *grey bars* represent intervals containing a recombination event. RLP50S values significantly different from the RLP50S value on the partially resistant line Vada are underlined ($4.43<LSD_{0.05}<8.38$). Missing values are indicated by a *. The number of recombinants identified between two consecutive markers is indicated within each marker interval on the chromosome bar. The markers in bold were used to identify recombination events within the L94 introgressions of Vada-*rphq2* and Vada-*rphq2*^{b}. The putative positions of *Rphq2* and *MlLa* are indicated by solid areas on the chromosome bar.

between sub-NILs carrying different alleles of *Rphq2* was equal to 14 corresponding to a prolongation of approximately 26 hours. QTL-mapping with the phenotypic and genotypic data



of the sub-NIL population also indicated P15M51-204, P14M50-225 and P14M54-252 as peak markers for *Rphq2* (Fig. 6). Two EST-based markers, WBE114 and WBE115, flanked these three AFLPs in a genetic interval of about 0.11 cM that corresponds to a rice syntenic stretch of sequence of 31.4 to 69.7 kbp. The marker order in the sub-NIL population agrees with the one on the L94 × 'Vada' map (Fig. 5a,b) and no inconsistency was found between the phenotype and the genotype of the 39 sub-NILs (Fig. 6).

The powdery mildew resistance gene *MlLa* also segregated in the 39 sub-NILs. On the basis of the infection types (Fig. 7), *MlLa* was fine-mapped distal to the microsatellite GBMS216 within the 1.2 cM chromosome segment differing between Vada-*rphq2* and Vada-*rphq2*^{b} (Fig. 6). Additional marker analyses explained the IT of 0.0 observed on v010101 by the presence of a remaining L94 fragment carrying the *mlo* gene that segregated in the BC_4S_1 generation of Vada-*rphq2*^{b} (data not shown). The genetic distance between the two AFLP markers flanking *mlo*, among the 157 AFLP markers used to select the Vada-NILs, was 28 cM. This is one of the biggest gaps observed between two adjacent AFLP markers used to select the Vada-NILs. The NILs and 38 other sub-NILs did not have this unwanted L94 fragment on chromosome 4HL.

Figure 7. First leaf of barley seedlings from the susceptible control 'Manchuria' (**a**) and the NIL Vada-*rphq2* (**b**) showing an IT of 4, from the cultivar 'Vada' (**c**) and the sub-NIL v40102 (**d**) showing an IT of 3 characteristic of the avirulence to *MlLa*, and from the line L94 (**e**) showing an IT of 0 characteristic of the presence of *mlo*. The pictures were taken 12 days after inoculation with *B. graminis* isolate C15.

Discussion

Verification of QTLs for partial resistance to *P. hordei* in NILs of barley

Recent technical advancements and refinements of analytical methods have enabled the molecular dissection of loci responsible for the genetic control of quantitative traits (Salvi and Tuberosa 2005). Nevertheless, the reliability at which a QTL effect can be assessed is the cornerstone towards the map-based isolation of the underlying gene(s). Out of the six QTLs for partial resistance to barley leaf rust identified by Qi et al. (1998b), the three loci explaining most of the variation (i.e. more than 15% per QTL) have been introduced into NILs and reciprocal NILs (Fig. 1). Compared with the results obtained on the RIL population (Qi et al. 1998b), our data on NILs confirm the substantial effect of *Rphq2* on LP of *P. hordei* and the lack of effect of *Rphq4* in the seedling stage (Table 1). In the RIL population the effect of *Rphq2* had been assessed as 35% of the phenotypic variation, whereas in the NILs this proportion appeared to be about 50%. The consistent and robust nature of *Rphq2* in spring barley was confirmed by its appearance in a recent linkage disequilibrium (association) mapping study on West-European spring barleys (Kraakman et al. 2006) and its detection in the mapping population derived from a cross between SusPtrit and 'Vada' (Jafary et al. 2006a). In the RILs, the phenotypic effect of *Rphq3* was stronger than observed in NIL backgrounds (Table 1). Apparently, the size of the effect of *Rphq3* depends on interaction with other genes, in particular *Rphq2*. Indeed, the RLP on Vada-*rphq3* was similar to the RLP on 'Vada', indicating the absence of effect of *rphq3* alone, while the RLP on Vada-*rphq2+3* was lower than the RLP on Vada-*rphq2* (Table 1), indicating a substantial effect of *rphq3* in presence of *rphq2*. This was confirmed by the proportion of early aborted colonies and the length of colonies microscopically measured on those NILs (Fig. 3). It is surprising that this interaction was not detected in the RIL population, in which Qi et al. (1998b) instead reported an interaction between *Rphq1* and *Rphq2*.

Partial resistance is due to a prehaustorially acting mechanism and is not based on hypersensitivity (Niks 1982, 1983a, 1986). But the confirmation that individual genes for partial resistance fit to these criteria has never been demonstrated to our knowledge. The percentage of early aborted colonies on L94-*Rphq2* was three times higher than the percentage of early aborted colonies on L94, while the percentage of early aborted colonies on L94-*Rphq3* was similar to that on L94 (Fig. 3). Depending on the genotype, a varying proportion of colonies were associated with host cell necrosis. Nevertheless, the necrosis occurred frequently as only one cell in relatively large established colonies, and was not particularly strong in 'Vada' or NILs carrying *Rphq2* and *Rphq3*. The fact that 69% of the early aborted colonies on L94-*Rphq2* were not associated with necrosis confirms that *Rphq2* confers predominantly a pre-haustorial type of resistance to *P. hordei*.

In conclusion, only *Rphq2* has a clear effect in the logistically convenient seedling stage and is an ideal candidate for map-based isolation experiments at this plant stage. However, the phenotypic effects of *Rphq3* and *Rphq4* in L94 genetic background were confirmed at the adult plant stage in repeated field trials (this thesis, Chapter 5), making these two QTLs possible targets for map-based isolation experiments at the, logistically less convenient, adult plant stage.

Construction of a high-resolution genetic map at the telomeric end of chromosome 2HL

RFLP probes have been used to construct the earliest barley linkage maps (Graner et al. 1991; Kleinhofs et al. 1993), which have then been used to integrate different barley genetic maps (Langridge et al. 1995; Qi et al. 1996; Karakousis et al. 2003; Marcel et al. 2007) and to anchor barley physical maps (Künzel et al. 2000; Stephens et al. 2004). However, RFLP markers are becoming too labour intensive and time-consuming to be used in large scale experiments and their conversion into locus specific PCR markers greatly helped the introgression of QTLs into the respective NILs. The converted RFLP markers were also decisive to estimate recombination frequencies within the QTL regions on the barley physical maps (Künzel et al. 2000; Stephens et al. 2004). On the basis of flanking RFLP-based markers, *Rphq2* was localised in a physical region of high recombination (i.e. 1 to 2 Mb/cM) (Table 2). Since *Rphq2* was also the easiest QTL to detect in seedlings (Table 1), it became our QTL of choice for map-based cloning. Two strategies were employed to further saturate the introgressions of L94-*Rphq2* and Vada-*rphq2* with molecular markers; a BSA approach and a synteny-based approach.

AFLP assays generate a high level of polymorphism and allow the simultaneous identification of a large number of amplification products. Those properties are suitable to identify molecular markers within a restricted region of the linkage map by BSA. Following this strategy, we successfully identified 13 AFLP markers within the 4.6 cM 'Vada' segment introgressed into L94-*Rphq2* after testing 48 *Pst*I/*Mse*I primer combinations. This number of markers identified is about as expected on the basis of the number of markers found by Qi et al. (1998a) in the same DNA segment; they found 6 AFLP markers after running 25 *Eco*RI/*Mse*I primer combinations. Three of the AFLP markers identified by BSA co-segregated with *Rphq2* (Fig. 6). Those markers are of utmost interest to identify BAC clones and to construct a physical map spanning *Rphq2*. The conversion of these markers into locus specific PCR markers will facilitate this process.

A syntenic relationship between rice chromosome 4 and the long arm of barley chromosome 2H has been reported by several authors (Moore et al. 1995; Schmierer et al. 2003; Devos 2005). This syntenic relationship was confirmed in our study in which seven barley ESTs identified by homology to predicted rice genes from four chromosome 4 BAC

clones mapped in the vicinity of *Rphq2* (Fig. 5). Micro-colinearity among the regions in rice and in barley was well conserved. Nevertheless, an inversion was observed in the order of the EST-based markers WBE110 and WBE113. Many studies reported such small translocations while saturating regions of barley leaf rust resistance genes *Rph7*, *rph16* and *Rph5*, respectively (Brunner et al. 2003; Perovic et al. 2004; Mammadov et al. 2005). Other reports indicated more complex deviation from micro-colinearity during fine-mapping QTLs for malting quality and for resistance to Fusarium head blight (Han et al. 1998; Liu et al. 2006).

Finally, the 36 molecular markers identified in the target region surrounding *Rphq2* were used to genotype a population of 39 sub-NILs and to generate a high-resolution genetic map of the region.

Feasibility of cloning *Rphq2*

Rphq2 is located on chromosome 2HL in the 2L1.0 region. This region was described by Dilbirligi et al. (2005) as a gene-rich region with a high level of similarity between wheat and barley. Within the 2L1.0 region of barley, the region of *Rphq2* was described as having the highest amount of recombination events (Dilbirligi et al. 2005). Still, the amount of recombination observed in our segregating population of 923 BC_4S_3 plants was two-fold reduced compared to the amount of recombination observed earlier in the RIL population.

The LP on the 39 sub-NILs was estimated two times on five seedlings per line. We needed data from those two experiments to separate two significantly distinct groups for LP that unambiguously placed the locus of the gene responsible for the QTL effect between markers WBE114 and WBE115 (Fig. 6). The assessment of the effect of *Rphq2* is not as straightforward as it is for major disease resistance genes that typically inherit according to Mendelian ratios. Yet, we anticipate that the amplitude of the effect of *Rphq2* is large enough to pursue the map-based cloning procedure.

Rphq2 was fine-mapped as a single, incompletely dominant gene, in a 0.11 cM genetic interval flanked by two EST-based markers (i.e. WBE114 and WBE115). In rice, the homologues of WBE114 and WBE115 delimit a physical interval of 31.4 to 69.7 kb. The incertitude is due to WBE114 being the best blastn hit for three different predicted rice genes encoding peroxidase proteins on the BAC OSJNBa0039K24. In barley, the megabase/centimorgan relationship in the syntenic interval is estimated at 1.1 to 1.8 (Table 2), suggesting a physical interval of 121 to 198 kb. Thus, it should be relatively easy to build a BAC contig and to close the gap between WBE114 and WBE115 using a BAC library. Two of such libraries are already available in barley for cultivars ‘Steptoe’ and ‘Cebada Capa’ (Yu et al. 2000; Isidore et al. 2005). Nevertheless, to maximise the chance of physically identifying the gene underlying *Rphq2*, we are currently constructing a new BAC library from the partially resistant cultivar ‘Vada’.

Identification of candidate genes in rice and in barley to explain *Rphq2*

Knowledge of the biological role of genes underlying QTLs for disease resistance remains limited and at present the only clue is obtained by the identification of candidate genes that co-incide with the QTLs. Two broad classes of genes concerned with plant defence are those involved in the recognition process and those involved in the defence-response process. It has been reported that modification of a monogenic race-specific resistance gene can give rise to a partial resistance gene (Li et al. 1999). After inspection of all predicted genes described in the refined *Rphq2* syntenic interval in rice, we did not find any homologue of such a major disease resistance gene. However, in the same syntenic target interval, genes involved in the defence-response process were identified. Six predicted rice genes on OSJNBa0039K24 encode for peroxidase proteins, three of which are homologous to WBE114. Genes of this peroxidase cluster might be candidates to explain *Rphq2*. Several authors already reported the association between peroxidase genes and loci for quantitative resistance (Faris et al. 1999; Ramalingam et al. 2003). Recently, the peroxidase gene locus *Prx2* was identified as a candidate to explain *Rphq2* (Marcel et al. 2007). We also identified in the rice syntenic region another predicted gene with high sequence similarity to a mitogen-activated protein kinase kinase kinase (MAP3K). Members of the MAP3K family are crucial for early defence signalling and cellular stress response to bacterial and fungal pathogens (Asai et al. 2002). Nevertheless, the gene responsible for *Rphq2* effect in barley might also have no counterpart in rice. A wheat-rice comparative genomics analysis indicated that gene evolution occurs preferentially at the ends of chromosomes (See et al. 2006). Telomeres are hot spots for all types of recombination. As a consequence, chromosomes loose synteny from each other at a faster rate in such high-recombination regions. Moreover, rearrangements in resistance gene regions that reduce microsynteny has often prevented the straightforward identification of a candidate gene by proxy (Brueggemann et al. 2002; Brunner et al. 2003; Perovic et al. 2004).

In barley, the *Rphq2* locus has been transferred to European cultivars from the 'botanical' barley line '*Hordeum laevigatum*' together with the tightly linked race-specific resistance genes *MlLa* and *Rdg1a* for resistance to powdery mildew and to leaf stripe, respectively (Giese et al. 1993; Arru et al. 2002). In this study, *MlLa* mapped distally from *Rphq2* ruling out the possibility that the same gene is responsible for quantitative resistance to leaf rust and qualitative resistance to powdery mildew. Arru et al. (2002) mapped *Rdg1a* about 10 cM proximal from *Rphq2*. In a leaf stripe disease test, L94-*Rphq2* was also as susceptible as the susceptible parent L94 (N. Pecchioni, personal communication) excluding as well the possibility that *Rphq2* and *Rdg1a* are the same gene. In barley, about 16 race-specific resistance genes for leaf rust (designated as *Rph* loci) have been reported (Franckowiak et al. 1997). None of them mapped on the long arm of chromosome 2H (Qi et al. 1998b). Nevertheless, a major gene for resistance to leaf rust has been detected at the distal

end of chromosome 2HL in an introgression from the bulbous barley grass *H. bulbosum* (Pickering et al. 2000). It would be interesting to determine whether the *H. bulbosum* resistance to barley leaf rust is allelic to *Rphq2* or not. In another study, Jafary et al. (2006a) mapped a QTL for resistance to a heterologous rust species, *P. persistens*, at the same position as *Rphq2*. This suggests the interesting possibility that this QTL determines basal resistance not only to *P. hordei*, but also to one or more rust taxa to which barley is a (near) non-host species.

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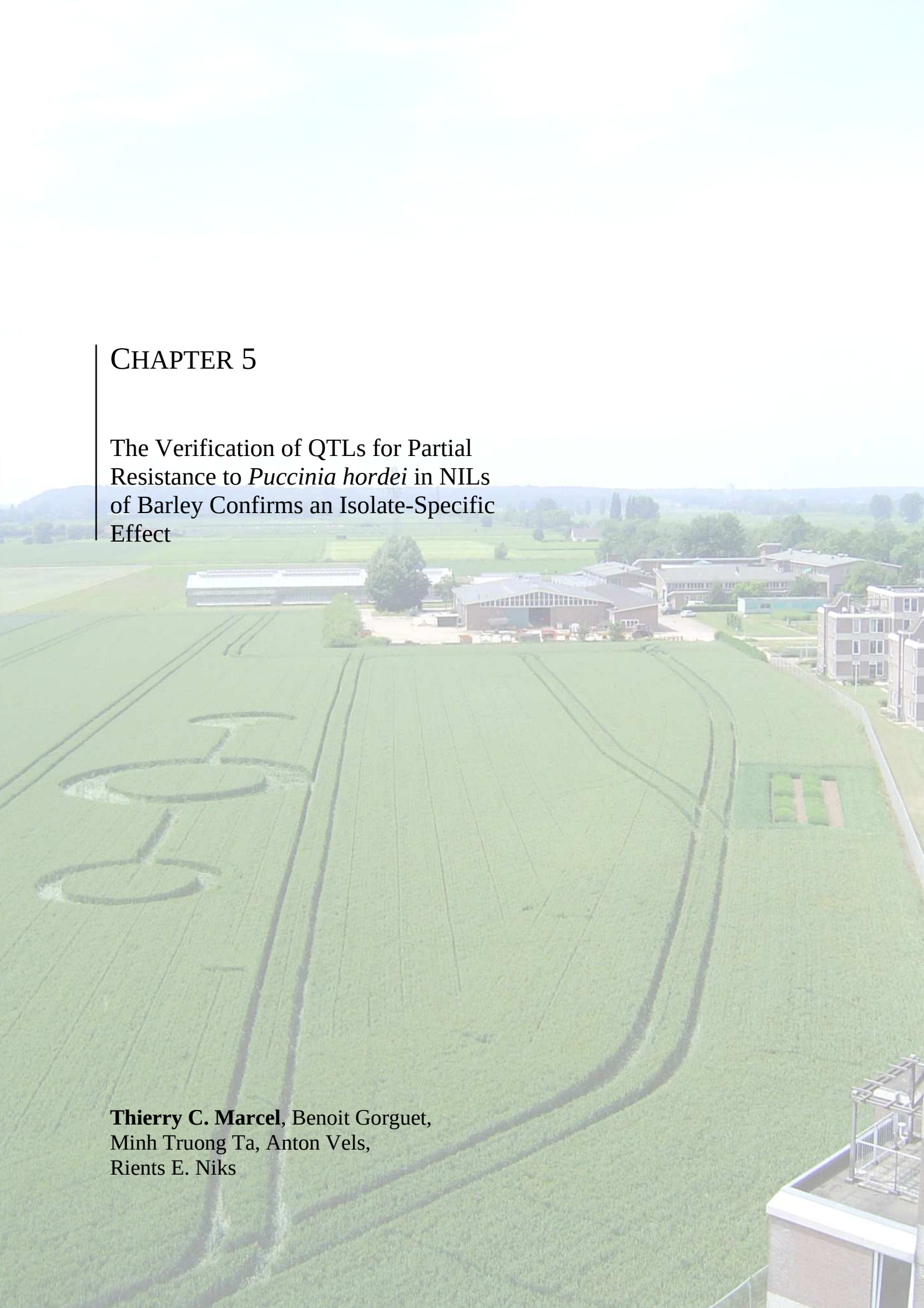
CHAPTER 5

The Verification of QTLs for Partial Resistance to *Puccinia hordei* in NILs of Barley Confirms an Isolate-Specific Effect



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Experimental field in 2004

The verification of QTLs for partial resistance to *Puccinia hordei* in NILs of barley confirms an isolate-specific effect

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Abstract Partial resistance is characterised by a reduced rate of epidemic development despite a susceptible infection type. Partial resistance typically inherits polygenically, each gene adding to the level of resistance. Partial resistance is considered race-non-specific and durable, fitting the concept of 'horizontal' resistance. However, detailed observations of the partial resistance to leaf rust (*Puccinia hordei* Otth) in barley (*Hordeum vulgare*) revealed small cultivar × isolate interactions suggesting a minor-gene-for-minor-gene interaction model, similar to the so-called 'vertical' resistance. Three consistent quantitative trait loci (QTL) that were detected in the cross susceptible L94 × partially resistant 'Vada' have been incorporated separately into L94 background to obtain near isogenic lines (NILs). Three isolates were used to map QTLs on seedlings of the L94 × 'Vada' population and to evaluate the effect of each QTL on adult plants of the respective NILs under field conditions. *Rphq2* had a strong effect in seedlings but almost no effect in adult plants against all the isolates tested, confirming previous results indicating that *Rphq2* is plant stage dependent, while *Rphq3* was effective in seedlings and in adult plants to all three isolates. However, *Rphq4* was effective in seedlings and in adult plants to two isolates but ineffective in both development stages to the third one, demonstrating a clear and reproducible isolate-specific effect. The resistance governed by the three QTLs was not associated with a hypersensitive reaction. Those results confirm the minor-gene-for-minor-gene model suggesting specific interactions between QTLs for partial resistance and *P. hordei* isolates.

Additionnal keywords: virulence spectrum; linkage map; QTL mapping; synergistic interaction

Introduction

Plants have developed different resistance strategies to protect themselves against invading pathogens. Such resistance can be qualitative and governed by one major gene or quantitative and governed by one to several minor genes, the so-called quantitative trait loci (QTLs). Resistance conferred by most major genes prevents fungal growth after the parasite has entered the host plant cell, and is accompanied by suicide of the penetrated cell or cluster of cells surrounding the site of challenge, a phenomenon known as hypersensitive response (HR). This HR-based resistance occurs upon direct or indirect recognition of a pathogen specific effector, known as avirulence (*Avr*) gene, by a host resistance (*R*) gene. Genetic variation in pathogen populations for *Avr* genes leads to race-specificity of this type of resistance. In partial resistance, minor genes typically stop fungal growth during the process of cell wall penetration (Niks and Rubiales 2002; Collins et al. 2007). Unlike in hypersensitive resistance, defended plant cells remain alive. Partial resistance was considered isolate-non-specific and durable, and therefore fitted Van der Plank's concept of 'horizontal' resistance (1963; 1968). However, detailed observations of the partial resistance to leaf rust in barley revealed small cultivar × isolate interactions (Parlevliet 1978; Parlevliet and van Ommeren 1985). Parlevliet and Zadoks (1977) explained these interactions by assuming a minor-gene-for-minor-gene interaction, similar to the so-called 'vertical' resistance. They even argued that the minor-gene-for-minor-gene interaction would explain the durability of this polygenic resistance (Parlevliet 2002).

More recently, Qi et al. (1999) and Niks et al. (2000a) mapped QTLs in the L94 × 'Vada' barley population against two and four different leaf rust isolates, respectively. Qi et al. (1998b, 1999) found that the three largest-effect QTLs were consistently effective against both isolates but seven small-effect QTLs were only effective against one of the two isolates tested, suggesting an isolate-specific effect. Niks et al. (2000a), however, found no evidence for isolate-specificity after testing this mapping population with four isolates. Isolate-specificity of QTLs has also been observed in other plant-pathosystems than barley-leaf rust (Leonards-Schippers et al. 1994; Caranta et al. 1997; Arru et al. 2003; Chen et al. 2003; Rocherieux et al. 2004).

Arru et al. (2003) suggested the existence in plants of separate gene classes conferring either race-specific or race-non specific resistance to different strains of pathogens. However, in all the studies reviewed, the QTLs consistently effective against all isolates tested were always those having the highest effect on resistance. The individual effects of genes controlling plant quantitative traits are often much smaller than the effects of the environment (Pooni and Kearsey 2002), underlining the importance of confirming a QTL effect across independent experiments. This also raises the question of the reliability of declaring small-effect QTLs isolate-specific while large-effect QTLs have not shown specificity so far. The

use of near isogenic lines (NILs) to test for isolate-specificity would allow to test simultaneously for the effect of several isolates and to use more replications since fewer plants are needed per experiment. With NILs, the effect of each QTL can also be determined without the interaction with other QTLs and the variable genetic background in the mapping population lines.

The aim of this research is to investigate whether larger-effect QTLs for partial resistance show specificity in their reactions when exposed to different isolates of barley leaf rust. NILs containing *Rphq2*, *Rphq3* and *Rphq4* (Van Berloo et al. 2001; Marcel et al. 2007) were tested with a set of 21 *P. hordei* isolates from which three were selected. The three selected isolates were used to map QTLs at seedling stage in the RIL population derived from the cross between L94 and 'Vada' and to test the NILs at adult plant stage under field conditions.

Material and methods

Plant and fungus material

A set of 103 F_9 recombinant inbred lines (RILs) derived from the cross between the leaf rust susceptible line L94 and the partially resistant cultivar 'Vada' was used to map QTLs for barley leaf rust resistance at seedling stage (Qi et al. 1998b). Through a marker-assisted backcross programme, Van Berloo et al. (2001) and Marcel et al. (2007) incorporated the QTLs *Rphq2*, *Rphq3* and *Rphq4* into L94 background to obtain NILs. L94-*Rphq2*, L94-*Rphq3* and L94-*Rphq4* contained 'Vada' introgressed fragments of 4.6, 22.6 and 10.8 centiMorgans (cM), respectively. The three NILs were evaluated at seedling stage in a greenhouse compartment and at adult plant stage in the field.

A set of 21 leaf rust isolates was

Table 1. Country of origin and collection date of 21 *Puccinia hordei* isolates

Isolate name	Origin	Collected
1.2.1[1, 2]	The Netherlands	1971
121-86	Monospore of 1.2.1	1986
3	Wales	1979
5.1	Israel	1979
9	Kenya	1977
13	Greece (Crete)	1979
17[1, 2]	The Netherlands	1973
18	The Netherlands	1974
22	France	1974
24[1]	The Netherlands	1974
25	Italy	1980
26[1, 2]	Finland	1980
28.1	Morocco	1981
29	Greece	1984
202	Israel	1976
Achterberg'01	The Netherlands	2001
Cordoba	Spain	1999
IVP2000	The Netherlands	2000
M7	Morocco	1986
Uppsala[1, 2]	Sweden	1999
Yellow mutant	Australia	unknown

[1] *P. hordei* isolates used for mapping QTLs on seedlings

[2] *P. hordei* isolates used for field experiments

Table 2. Differential series of barley lines carrying different *Rph*–genes

Line	*Rph*-gene symbol[1]	Previous name
L94	-	
Sudan	*Rph1.a*	*Rph1*
Peruvian	*Rph2.b*	*Rph2*
Estate	*Rph3.c*	*Rph3*
Gold	*Rph4.d*	*Rph4*
Magnif 102	*Rph5.e*	*Rph5*
Bolivia	*Rph2.r, Rph5.f*	*Rph2, Rph6*
Cebada Capa	*Rph7.g*	*Rph7*
Tunisian 17	*Rph7.ac, RphC*	
Egypt 4	*Rph8.h*	*Rph8*
Hor 2596	*Rph9.i*	*Rph9*
Trumpf	*Rph9.z*	*Rph12*
Tunisian 34	*RphD*	

[1] *Rph*-gene symbols are given according to the recommendation of Franckowiak et al. (1997), and to the allelic tests of Zhong et al. (2003) for *Rph5.f* and Borovkova et al. (1998) for *Rph9.z*

applied on seedlings of thirteen barley lines and cultivars to determine their virulence spectra (Table 1). Most of the thirteen barley accessions tested (Table 2) belong to the regular differential series for barley leaf rust (Clifford 1985; Niks et al. 2000b). The inoculation was performed as described in the next section and 10 to 12 days later, infection types (ITs) were scored according to the scale of McNeal et al. (1971) modified by Shtaya et al. (2006a). Lines with ITs 0–3 were regarded as resistant, 4–6 as moderately resistant and 7–9 as susceptible. All isolates were multiplied in separate greenhouse compartments on the susceptible barley line L98. Once collected, the spores were dried in a desiccator and stored at –80°C.

Disease evaluations at the seedling stage

Before inoculation, urediospores were taken from the –80°C freezer and thawed in 38–42°C water. The first leaf of each seedling was fixed horizontally on the soil, adaxial side up, and trays were placed in a settling tower. Then 3 mg of urediospores diluted 10 times with lycopodium spores were dusted over each tray, resulting in about 180 rust spores per cm^2. After incubation overnight (8 hours) at 100% relative humidity in a dark dew chamber at 18°C, the seedlings were transferred to a greenhouse compartment at 20±3°C with 30 to 70% relative humidity. The latency period (LP) on each seedling was evaluated by estimating the period (hours) at which 50% of the ultimate number of pustules became visible. The relative latency period of seedlings (RLP50S) was calculated relative to the LP of L94 seedlings, where L94 was set at 100, as described by Parlevliet (1975).

In a preliminary greenhouse evaluation, we evaluated the NILs with the twenty-one isolates of the pathogenic fungus *Puccinia hordei* (Table 1). Four seeds of each NIL and parent, L94 and 'Vada', were sown in trays of 37x39 cm. One tray per isolate was used, and three series of evaluation were necessary to test the twenty-one isolates. In each series the isolate 1.2.1 was used as a recurrent standard.

A subset of three *P. hordei* isolates, selected for their differential effects between NILs containing different QTLs, was used to map QTLs on seedlings of RILs. For each isolate four

seedlings per RIL and 24 seedlings of L94 and 'Vada' were evaluated in two consecutive experiments.

Disease evaluations in the field

The NILs were tested in the field in 2003 and 2004 against the three selected leaf rust isolates 17, 26 and Uppsala, and against our standard isolate 1.2.1. The trial design was a split-plot in two to four replicates, depending on seed supply, with isolates on main plots and barley lines on subplots. Replications within plots were arranged as blocks. Within each replication the order of the subplots was randomised. The main plots were separated from each other by a distance of 100-200 m cultivated with oat in 2003 and with winter wheat in 2004. Each subplot of a barley accession consisted of three rows (about 50 seeds per row) sown at 0.25 m interval and alternated with similar subplots of oat. Sowing in the field was done on 21–23 April for the two experiments. For each isolate, 20 pots containing five seeds of the susceptible genotype L98 were spray-inoculated in the greenhouse and incubated overnight at a relative humidity of 100%. Before sporulation of these spreader plants, on 1–2 June, the pots were placed uniformly in the field and removed eight days later. The climatic conditions were more favourable to initiate the epidemics in 2003 than in 2004. In 2003, three disease assessments were performed at 18, 23 and 29 days after placement of the spreader pots (dpi), and in 2004, four disease assessments were performed at 25, 30, 36 and 41 dpi for isolate Uppsala and 25, 36, 41 and 45 dpi for isolates 17, 26 and 1.2.1. Per assessment, three random tillers were sampled per subplot to count the number of mature rust pustules on the three upper leaves. After the last disease evaluation, spores of the four isolates were collected from the field. Their virulence spectrum was determined as described earlier and compared to the virulence spectrum of the isolates used in the greenhouse seedling tests.

A logarithm transformation (Ln-scale) was performed on the data collected according to the formula $T_{log}=\ln(P+1)$, where P = number of rust pustules, to satisfy the condition of homogeneity of variance. The transformed data were used to calculate the Area Under Disease Progress Curve (AUDPC) according to the formula $AUDPC = \sum (t_{(i+1)} - t_i) (y_{(i+1)} + y_i) / 2$, where t_i = first assessment date of two consecutive assessments, y_i = disease severity on assessment date t_i, $t_{(i+1)}$ = second assessment date of two consecutive assessments and $y_{(i+1)}$ = disease severity on assessment date $t_{(i+1)}$. A Duncan's multiple range test was performed with GenStat® Release 8.1 (VSN International Ltd. 2005) to compare all pairs of means.

Map construction and QTL mapping

A data set of 958 morphological and molecular markers segregating in the 103 RILs of L94 × 'Vada' was used to construct a dense marker map of the barley genome. This data set has

previously been used as a component of a high-density consensus map of barley (Marcel et al. 2007), and is predominantly composed of AFLPs (709 markers) and microsatellites (138 markers). JoinMap® 3.0 (Van Ooijen and Voorrips 2001) was used for linkage grouping and map construction. Linkage groups were assigned to the corresponding barley chromosomes according to previously published maps (Qi et al. 1998a; Marcel et al. 2007). Map distances were calculated using the Kosambi's mapping function. A skeletal map with 210 uniformly distributed markers (approximately 5 cM per marker interval) was extracted. All the markers on the skeletal map fitted in the dense map during the first or exceptionally the second round of JoinMap® 3.0. This skeletal map was used for QTL analyses in a previous study by Shtaya et al. (2006b) and in the present study.

The wide sense heritability (h^2) for RLP50S was estimated from ANOVA according to the formula $h^2 = \sigma^2_g /(\sigma^2_g + \sigma^2_e/n)$ with the genetic variance $\sigma^2_g=(MS_g-MS_e)/n$, the environmental variance $\sigma^2_e= MS_e$, *MS* the mean square and n the number of replicates per RIL. ANOVA on RLP50S revealed significant genotype and replication effects with all *P. hordei* isolates tested. Therefore, the genotype effect of each line was extracted from the analysis of variance and used to map QTLs on the skeletal map. The ANOVA was performed with the GenStat® 8.1 software package (VSN International Ltd. 2005). QTL mapping was performed using MapQTL® 5.0 (Van Ooijen 2004). Interval Mapping (IM) was run and, in the region of the putative QTLs, the markers with the highest LOD values (peak markers) were used as co-factors for running a multiple-QTL mapping programme, the MQM method (Jansen and Stam 1994). When LOD values of some markers on other regions reached the significance level, the MQM was repeated by adding those new 'peak markers' as co-factors until a stable LOD profile was reached. The restricted-MQM method (rMQM) was used to determine the values of the LOD, phenotypic variation, additive effect and the confidence interval for the detected QTLs. After a genome-wide permutation test on each set of data, an LOD threshold value of 2.9 for *P. hordei* 1.2.1 and 26, of 3.0 for *P. hordei* 17 and Uppsala and of 3.1 for *P. hordei* 24 was set for declaring a QTL.

Results

Characterisation of the 21 barley leaf rust isolates

The 21 leaf rust isolates were classified according to their virulence/avirulence pattern on seedlings of the differential series of barley accessions and for their latency period on the NILs with individual *Rphq*-genes. This virulence characterisation distinguished sixteen races of *P. hordei* (Table 3). None of the resistance genes was effective to all *P. hordei* isolates because isolate 28.1 was virulent to all resistance genes tested. The results of this survey helped to control the identity of the isolates tested later in the field.

Table 3. Resistance/Susceptibility pattern[1] of 21 *P. hordei* isolates according to their infection type on a differential series of barley lines carrying different *Rph*-genes and classification of the same isolates according to their latency period on QTL near isogenic lines

	RphD	*Rph7.g*	*Rph7.ac, RphC*	*Rph3.c*	*Rph9.i*	*Rph9.z*	*Rph5.e*	*Rph8.h*	*Rph2.r, Rph5.f*	*Rph2.b*	*Rph1.a*	*Rph4.d*	Race[2]	Class[3]
28.1	S	S	S	S	S	S	S	S	S	S	S	S	1	2 > 3
M7	R	S	R	S	S	MR	S	S	S	S	S	S	2	2 > 3
13	R	R	S	R	MR	S	S	S	S	S	S	S	3	2 = 3
Uppsala	R	R	S	R	MR	MR[4]	MR	S	S	S	S	S	4	2 = 3
18	R	R	R	S[4]	S	S	S	S	S	S	S	S	5	2 > 3
Achterberg'01	R	R	R	S	S	S	R	R	S	S	S	S	6	2 = 3
9	R	R	R	S	MR	S	S	S	S	S	S	S	7	2 > 3
5.1	R	R	R	S	MR	S	S	S	S	S	S	S	7	2 = 3
202	R	R	R	S	MR	R	S	S	R	S	S	S	8	2 > 3
IVP 2000	R	R	R	R	S	S	S	S	S	S	S	S	9	2 > 3
17	R	R	R	R	S	S	S	S	S	S	S	S	9	2 = 3
121-86	R	R	R	R	S	S	S	S	S	S	S	S	9	2 = 3
25	R	R	MR	R	S	S	S	S	S	S	S	S	9	2 = 3
26	R	R	R	R	S	S	MR[4]	S	S	S	S	S	10	2 < 3
Cordoba	R	R	R	R	S	S	R	MR	S	S	S	S	11	2 = 3
29	R	R	R	R	S	R	S	MR	S	S	S	S	12	2 > 3
3	R	R	R	R	S	MR	S	S	S	S	S	S	13	2 < 3
1.2.1	R	R	R	R	S	MR	S	S	S	S	S	S	13	2 > 3
Yellow Mutant	R	R	R	MR	MR	S	MR	S	S	S	S	S	14	2 > 3
24	R	R	R	R	MR	S	MR	MR	S	MR	S	S	15	2 > 3
22	R	R	R	R	MR	MR	S	S	S	S	S	S	16	2 = 3

[1] Lines with infection types 0–3 are resistant (R); lines with infection types 4–6 are moderately resistant (MR); lines with infection types 7–9 are susceptible (S)

[2] Different races are distinguished by different virulence spectra

[3] 2 > 3: *Rphq2* prolongs LP more than *Rphq3*; 2 = 3: *Rphq2* and *Rphq3* prolong LP to similar extent; 2 < 3: *Rphq2* prolongs LP less than *Rphq3*

[4] Different seedlings showed contrasting infection types

The RLP50S on NILs containing *Rphq2*, *Rphq3* and *Rphq4* distinguished three classes of isolates (Table 3). A first class for which the RLP50S on L94-*Rphq2* was higher than the RLP50S on L94-*Rphq3* (10 isolates), a second class for which the RLP50S on L94-*Rphq2*

was about similar to the RLP50S on L94-*Rphq3* (9 isolates) and a third class for which the RLP50S on L94-*Rphq2* was lower than the RLP50S on L94-*Rphq3* (2 isolates). Qi et al. (1998b, 1999) reported that *Rphq4* is ineffective in seedlings to isolates 1.2.1 and 24. However, Uppsala had a significantly higher RLP50S on L94-*Rphq4* than on L94 (Table 4). Based on those observations, the leaf rust isolates 17, 26 and Uppsala were selected for further studies on the isolate-specificity of QTLs. Isolate 17 (second class) had a similar RLP50S on L94 as on the NILs (Table 4). However, the level of partial resistance of the cultivar 'Vada' against isolate 17 was still high, indicating that the resistance of Vada to this isolate might be due to different QTL(s) than the ones that are effective against other isolates. Isolate 26 (third class) had a significantly higher RLP50S on L94-*Rphq3* than on L94 while the RLP50S on L94-*Rphq2* was similar as on L94 (Table 4), in contrast to isolate 1.2.1 (our standard isolate). Finally, isolate Uppsala, one of the most aggressive isolates tested, had a significantly higher RLP50S on L94-*Rphq3* and on L94-*Rphq4* than on L94 (Table 4). The virulence spectra of the three selected rust isolates were different, indicating distinct races (Table 3).

Table 4. Relative latency period of seedlings (RLP50S) of L94[1], Vada and NILs measured against the *P. hordei* isolates 1.2.1, 17, 26 and Uppsala

	1.2.1[2]	17	26	Uppsala
L94	100	100	100	100
L94-*Rphq2*	104*	100	102	102
L94-*Rphq3*	98	102	108*	104*
L94-*Rphq4*	96*	99	100	105*
Vada	128*	119*	125*	127*

[1] L94 is set at RLP50S = 100

[2] An *asterisk* indicates that the mean differs significantly from the mean of L94 ($LSD_{0.05}$)

Construction of a dense marker map of L94 × 'Vada'

The 958 markers segregating in L94 × 'Vada' were assembled into 7 linkage groups corresponding to the 7 barley chromosomes 1H to 7H, homeologous to the wheat chromosomes 1 to 7. The new L94 × 'Vada' dense marker map had a total map length of 1,088 cM with an average distance between two consecutive loci of 1.1 cM. This represents a substantial improvement compared to the previously published linkage map of L94 × 'Vada' that covered 1,062 cM with an average distance between two consecutive loci of 1.9 cM (Qi et al. 1998a). The distribution of 235 new *Pst*I/*Mse*I AFLP markers was more homogeneous compared to the distribution of the previous 561 *Eco*RI/*Mse*I AFLP markers (Marcel et al. 2007). The three gaps larger than 20 cM reported by Qi et al. (1998a) on chromosomes 1H, 3H and 7H have been reduced in this new map. Only one of them, on chromosome 1H,

remained larger than 10 cM. The average marker distance on the extracted skeletal map used for QTL mapping in this study was 5.2 cM. The map lengths and marker order were highly consistent with those of the high-density molecular map constructed by Qi et al. (1998a) and the high-density consensus map of barley constructed by Marcel et al. (2007). All the mapping data and segregation data of this new L94 × 'Vada' linkage map have been deposited in the GrainGenes 2.0 database (Barley, L94 x Vada, 2006).

QTL mapping confirms the isolate-specificity of small-effect QTLs in seedlings

To investigate the isolate-specificity of individual QTLs for partial resistance in seedlings, the L94 × 'Vada' segregating population was challenged with the three virulent *P. hordei* isolates 17, 26 and Uppsala (Table 1). Raw data obtained from Qi et al. (1998b, 1999) were available for isolates 1.2.1 and 24.

The wide sense heritability (h^2) for RLP50S was 0.84 with *P. hordei* 17, 0.88 with *P. hordei* 26 and 0.89 with *P. hordei* Uppsala. The RLP50S values of *P. hordei* 17 and 26 slightly exceeded the range between the susceptible line L94 and the partially resistant line 'Vada' (Fig. 1a,b), but those for *P. hordei* Uppsala did not (Fig. 1c).

The genotype effect was extracted from ANOVA and used for QTL mapping. A total of eight QTLs were detected with one or more of the leaf rust isolates tested (Table 5). The QTLs with a

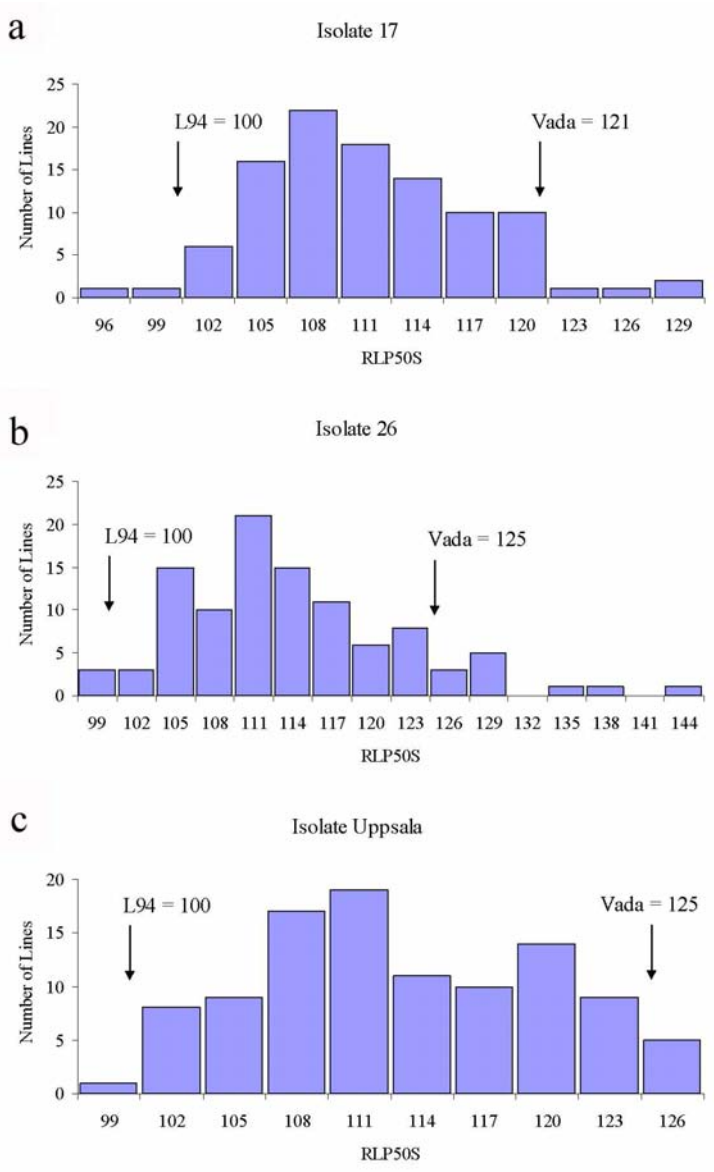

Figure 1. Frequency distribution of phenotypes for the measures of leaf rust resistance in seedlings (RLP50S) with (**a**) isolate 17, (**b**) isolate 26 and (**c**) isolate Uppsala, in 103 RILs derived from the cross L94 × 'Vada'. Values of L94 and 'Vada' are shown by an *arrow*. The values indicated on the *x*-axis are the average values of each category.

significant LOD value with IM and/or with rMQM (Table 5), explained together 59%, 39%, 56%, 60% and 72% of the phenotypic variance for isolate 1.2.1, 24, 17, 26 and Uppsala, respectively. The QTLs *Rphq2* and *Rphq3* explained most of the phenotypic variance with all isolates, while the other QTLs contributed moderately to the total level of partial resistance. The resistance allele of seven QTLs originated from the partially resistant parent 'Vada', while the resistance allele of *Rphq17* originated from the susceptible parent L94.

Three of the QTLs identified, *Rphq1*, *Rphq2* and *Rphq3*, were effective against all five isolates, confirming the results of Qi et al. (1998b) who first identified them against isolate 1.2.1. A fourth QTL, *Rphq7*, was effective to isolate 24 with a LOD value above the threshold (Table 5). At the same linkage group position as *Rphq7* was indicated, we also found consistent LOD peaks between 2 and 3 to the other four isolates (Table 5; Fig. 2). The four other QTLs, *Rphq4*, *Rphq17*, *Rphq20* and *Rphq21*, had an effect to only one or two of the three isolates tested in this study (Table 5). One QTL, *Rphq17*, was at a mapping position similar to a QTL reported in the Oregon Wolfe Barleys population (Marcel et al. 2007). We assume that it is at the same locus and provisionally use the same gene designation as Marcel et al. (2007). *Rphq20* and *Rphq21* were at locations in which no QTL for resistance to *P. hordei* had been reported before. Surprisingly, the QTLs having an effect to one or two rust isolates but not to the others were not detected with the IM method (LOD values below 2) while they were detected with LOD values up to 8.2 with the rMQM method (Fig. 2).

Synergistic interactions between QTLs corroborate an isolate-specific effect

The isolate-specific effect of *Rphq4*, *Rphq17*, *Rphq20* and *Rphq21* observed by QTL mapping was associated with strong discrepancies between the results obtained by IM and by rMQM methods (Fig. 2), questioning the reliability of declaring those QTLs isolate-specific. *Rphq17* and *Rphq20* had the greatest contrast between LOD values calculated by IM and by rMQM methods (Table 5; Fig. 2). *Rphq17* had only a significant effect to isolate 26 and *Rphq20* to isolate Uppsala but each QTL had also a peak LOD value between 2 and 3 to isolate Uppsala and 26, respectively.

We determined whether the presence of other QTLs in the genetic background had an influence on the effect of *Rphq17* to isolate 26 and of *Rphq20* to isolate Uppsala (Fig. 3a,b). Here, the effect of a QTL refers to the average difference of LP in hours between the RILs carrying the resistance allele and the RILs carrying the susceptibility allele at the LOD peak marker of that QTL. The presence of the resistance allele of *Rphq2*, *Rphq3*, *Rphq4* or even *Rphq20* increased the effect of *Rphq17* against isolate 26 from 2 (*Rphq2*) to 9-fold (*Rphq4*) when compared to the presence of the susceptibility allele of those QTLs (Fig. 3a). In a similar way, *Rphq20* had an effect against isolate Uppsala only in the presence of the resistance allele of *Rphq2* or *Rphq17*, and the resistance allele of *Rphq3* increased the effect

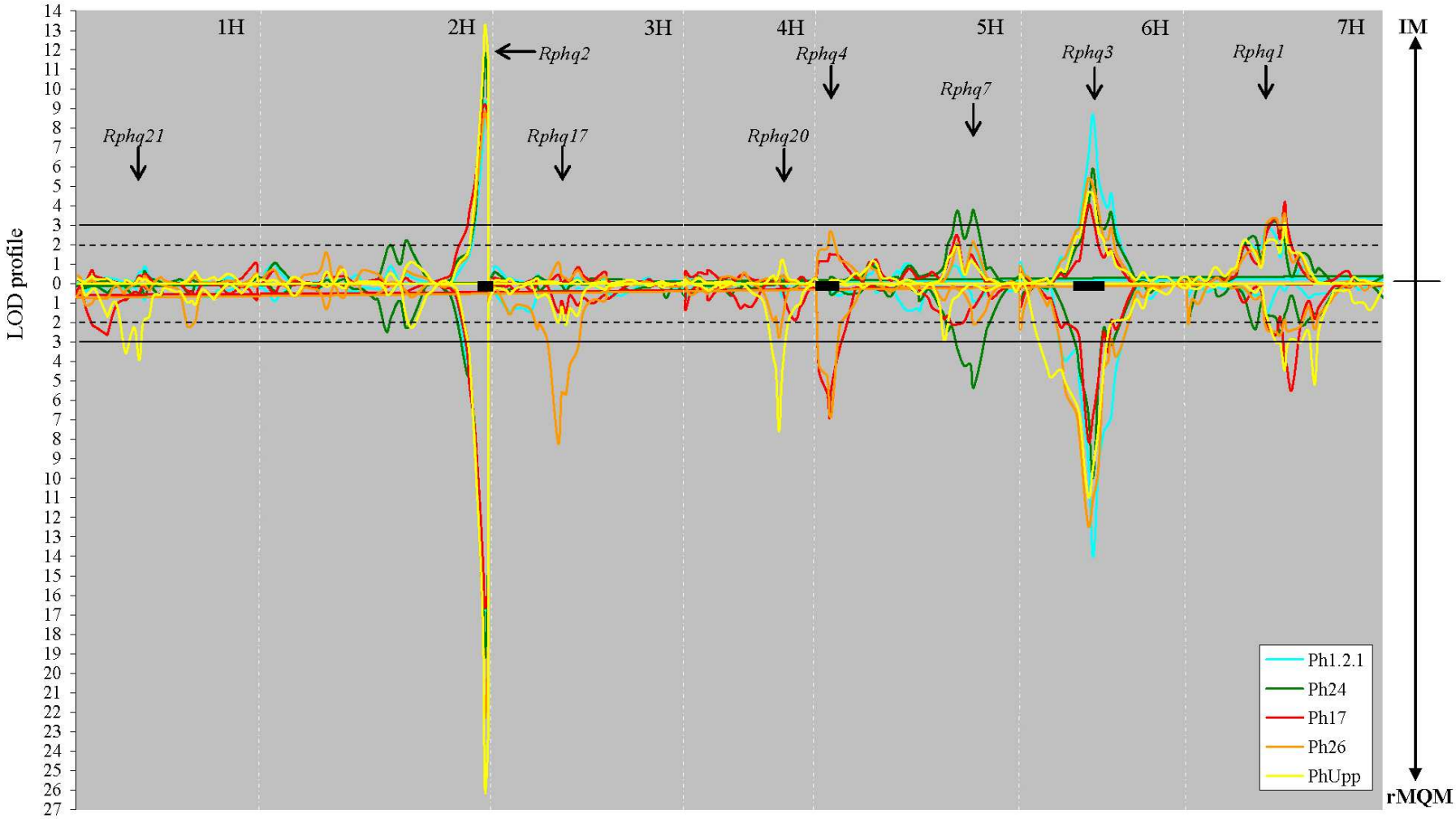

Figure 2. LOD profiles of five barley leaf rust isolates obtained by interval mapping (IM), above the *x*-axis, and by restricted multiple-QTL mapping (rMQM), below the *x*-axis, along the linkage map constructed with the L94 × 'Vada' RIL population. *Dashed vertical lines* separate the seven barley chromosomes. *Solid horizontal lines* indicate approximately the LOD threshold for significance and *dashed horizontal lines* a LOD of 2. Names of the identified QTLs are given with an arrow indicating the approximate position of their peak marker. The three boxes on the *x*-axis represent the 'Vada' fragments introgressed into L94 background to develop NILs.

Table 5. Summary of QTLs for partial resistance to five leaf rust isolates at seedling development stage

			P. hordei 1.2.1[1]		*P. hordei* 24[1]		*P. hordei* 17		*P. hordei* 26		*P. hordei* Upp.	
QTLs	Chr.	cM[2]	IM[3]	rMQM[4]	IM	rMQM	IM	rMQM	IM	rMQM	IM	rMQM
Rphq1	7H	46–122	**2.9**	1.6	**3.2**	2.5	**4.2**	**5.4**	**3.4**	2.6	**3.1**	**4.4**
Rphq2	2H	187–192	**9.4**	**17.1**	**11.8**	**19.1**	**10.8**	**16.7**	**9.2**	**22.3**	**13.3**	**26.1**
Rphq3	6H	53–63	**8.7**	**14.0**	**5.9**	**10.0**	**4.0**	**8.0**	**5.4**	**12.4**	**4.6**	**10.9**
Rphq4	5H	5–16	–[5]	–	–	–	1.5	**6.9**	2.7	**6.8**	–	–
Rphq7	5H	101–138	2.1	0.9	**3.8**	**5.3**	2.5	2.1	2.1	2.1	2.0	**3.0**
Rphq17[6]	3H	54–60	–	–	–	–	–	–	0.3	**8.2**	0.0	2.1
Rphq20	4H	76–79	–	–	–	–	–	–	0.4	2.8	1.3	**7.6**
Rphq21	1H	36–56	–	–	–	–	–	–	–	–	1.3	**3.9**

[1] Raw data obtained from Qi et al. (1998b; 1999)

[2] Position of the two-LOD confidence interval based on the results of rMQM mapping on L94 × 'Vada' marker map

[3] LOD value obtained with the interval mapping method; values in bold are above the LOD treshold

[4] LOD value obtained with the restricted-MQM method; values in bold are above the LOD threshold

[5] Data are presented only when LOD ≥ 2 with IM or with rMQM

[6] The resistance allele was contributed by L94

of *Rphq20* more than 3-fold when compared to the presence of the susceptibility allele of this QTL (Fig. 3b). However, the presence of the 'Vada' allele of *Rphq4* did not influence the effect of *Rphq20* against isolate Uppsala. In this study, *Rphq4* was only detected on seedlings challenged with isolates 17 and 26. The fact that *Rphq4* has no influence on the effect of

Rphq20 when challenged with isolate Uppsala is consistent with the fact that *Rphq4* is not effective against that isolate. The interactions observed confirm an effect of *Rphq17* against isolate 26 and an effect of *Rphq20* against isolate Uppsala. Those results corroborate the isolate-specific effect of *Rphq4*, *Rphq17* and *Rphq20*, and suggest a synergistic interaction between any pair of QTLs expressed in a same genetic background.

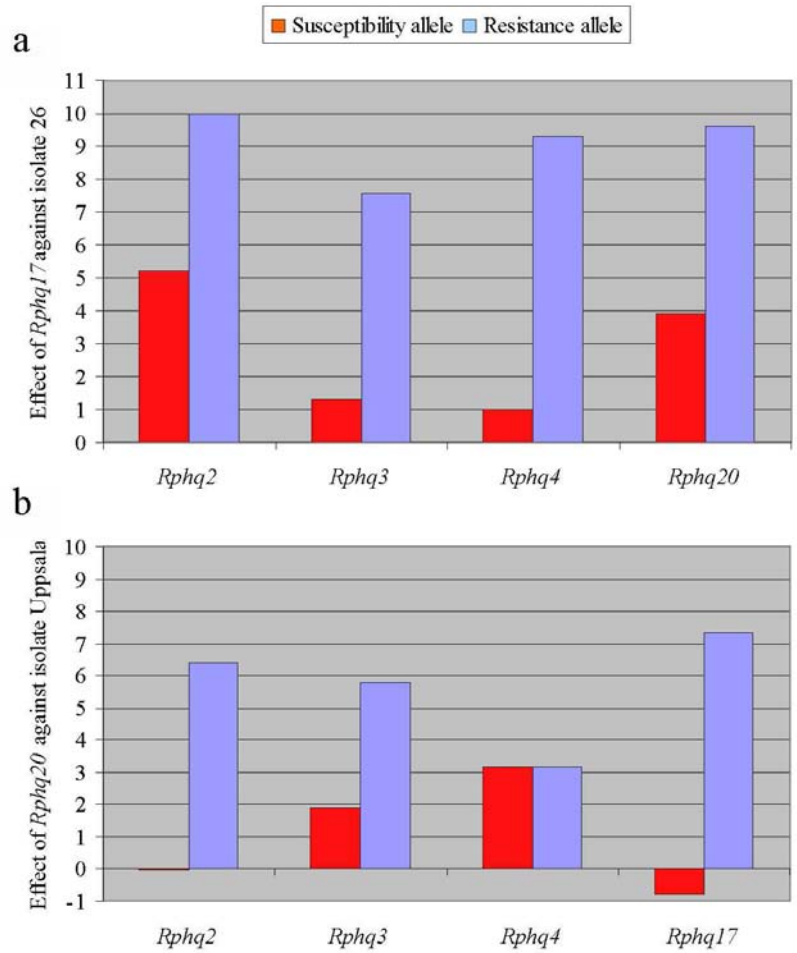

Figure 3. (**a**) The effect of the resistance allele of *Rphq17* (prolongation of LP in hours) against isolate 26 in the genetic background of RILs having the susceptibility or the resistance allele of *Rphq2*, *Rphq3*, *Rphq4* or *Rphq20*, and (**b**) for *Rphq20* against isolate Uppsala in the genetic background of RILs having the susceptibility or the resistance allele of *Rphq2*, *Rphq3*, *Rphq4* or *Rphq17*.

The isolate-specificity of *Rphq4* is confirmed under field conditions

The NILs with individual *Rphq*-genes were used to verify the QTLs under agricultural conditions. The three selected isolates, 17, 26 and Uppsala, and the isolate 1.2.1 were used to determine the effect of *Rphq2*, *Rphq3* and *Rphq4* in their NIL-background in the field. This experiment was conducted two times, in 2003 and in 2004.

Against isolate 1.2.1, *Rphq3* and *Rphq4* had a significant effect in 2003 and 2004, while *Rphq2* had a significant effect in 2004 only (Fig. 4a,b). The effect of *Rphq3* was always stronger than the one of *Rphq4*. Those results do not agree with the ones of Qi et al. (1998b), who found that, on adult plants of the mapping population, *Rphq4* was the most effective

QTL. This disagreement indicates that *Rphq4* is not consistently expressed in the RILs and in its NIL-background. Against isolates 17 and 26, *Rphq2* did not always have a significant effect, while *Rphq3* and *Rphq4* always had a clear and significant effect on the level of partial resistance (Fig. 4c,d,e,f). Against isolate Uppsala, however, the only QTL having a significant effect in 2003 was *Rphq3* (Fig. 4g), while none of the QTLs had a significant effect in 2004 (Fig. 4h). Nevertheless, L94-*Rphq3* was the NIL with the lowest AUDPC against isolate Uppsala in 2004 (Fig. 4h).

As expected, the partially resistant 'Vada' had the lowest AUDPC with all four isolates (Fig. 4). The AUDPC of L94-*Rphq2* was significantly lower than the AUDPC of L94 against one isolate in 2003 (26; Fig. 4e) and against two isolates in 2004 (1.2.1 and 17; Fig. 4b,d), indicating that the effect of *Rphq2* introduced in L94 background is weak in adult plants, and that different environmental conditions can influence its phenotypic expression. The AUDPC of L94-*Rphq3* was significantly lower than the AUDPC of L94 against the four isolates in 2003 and against three isolates in 2004. The AUDPC of L94-*Rphq4* was always significantly lower than the AUDPC of L94 against isolates 1.2.1, 17 and 26 but not significantly different against isolate Uppsala, demonstrating the isolate-specificity of *Rphq4* at the adult plant stage and confirming the results obtained earlier by QTL mapping on seedlings.

In the field, the contamination of one or several isolates by another local isolate could dramatically change the results and their interpretation. To confirm their identity, samples of the four isolates were collected in the field after the last disease assessment of 2003 (data not presented). Seven barley lines from the differential series evaluated earlier were chosen to compare the virulence spectra of the four isolates. The infection types of the collected isolates corresponded to the infection types observed earlier on the seedlings (Table 3). Because the infection types were similar, the contamination of the experiment by a local leaf rust isolate is not likely. In commercial barley fields in Wageningen, barley leaf rust is indeed not an abundantly occurring pathogen.

Discussion

Since Parlevliet and Zadoks (1977) proposed and discussed the hypothesis of minor-gene-for-minor-gene interactions, it partly remained an open question whether individual QTLs for partial resistance can be effective to some races of a pathogen and ineffective to others. Several authors mapped QTLs in a segregating population with different isolates of a same pathogen (Qi et al. 1999; Niks et al. 2000a; Arru et al. 2003; Chen et al. 2003; Rocherieux et al. 2004). In each case, they found that the QTLs that were effective against all the isolates were always those with the highest effect on resistance. The present research was aimed to determine whether larger-effect QTLs for partial resistance to leaf rust in barley may show specificity in their reaction to different *P. hordei* isolates.

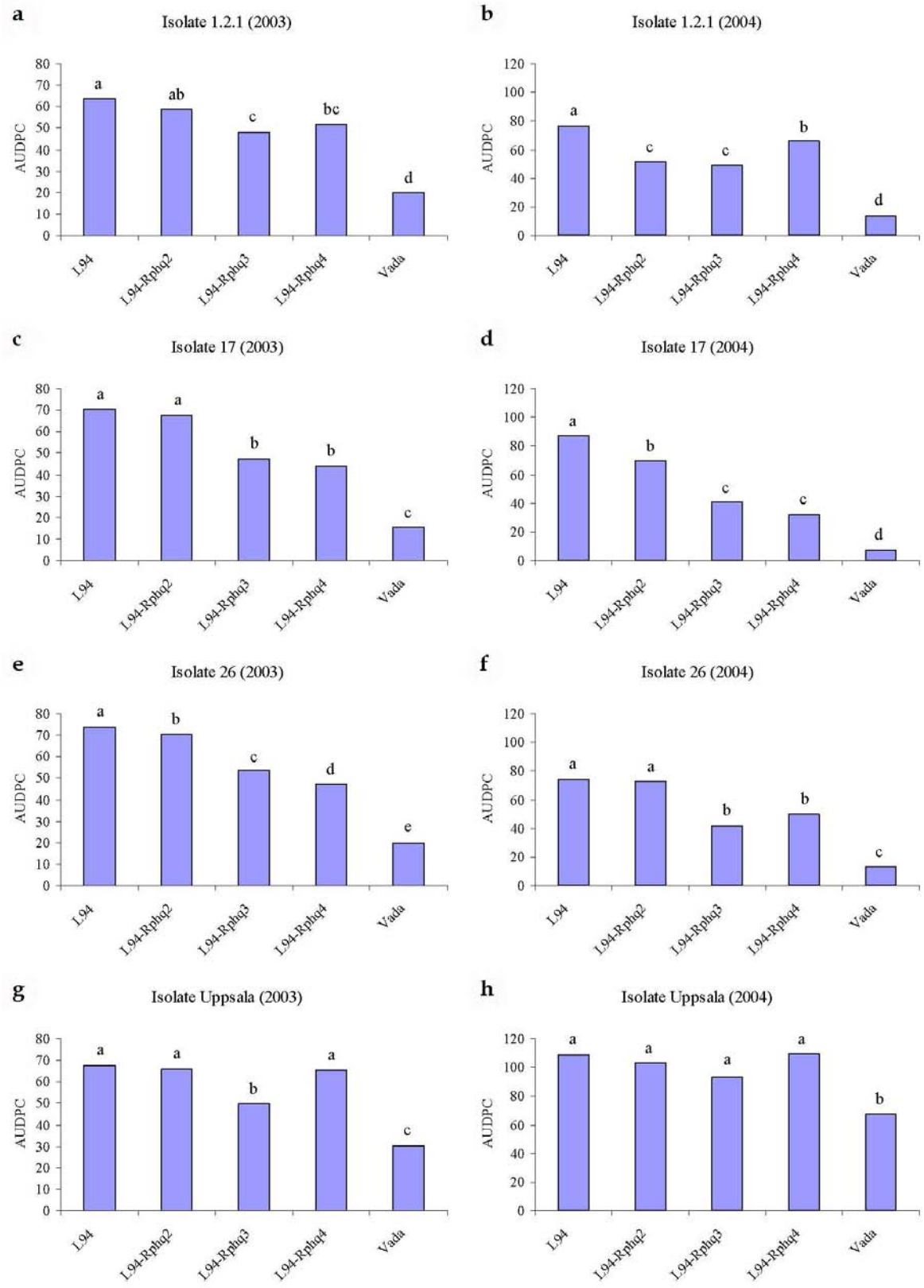

Figure 4. Histograms of AUDPC of barley accessions L94 and 'Vada' and of the NILs L94-*Rphq2*, -*Rphq3* and -*Rphq4* tested against the leaf rust isolates 1.2.1, 17, 26 and Uppsala, during the field evaluations of 2003 and 2004. Similar letters on bars indicate that the means do not differ significantly according to Duncan's test ($P < 0.05$).

We determined the virulence spectra of 21 isolates on a barley differential series and their latency period on near-isogenic lines that each carried a different minor gene for partial resistance (Table 3). The virulence spectra for isolates, as far as tested before, were generally in agreement with earlier data (Parlevliet et al. 1981; Niks et al. 1989). Our data indicate different specificities for *Rph7.g* (Cebada Capa) and *Rph7.ac* (Tunisian 17), which belong to a multiple allelic series mapped on the short arm of chromosome 3H (Chicaiza et al. 1996; Graner et al. 2000; Isidore et al. 2005). Interestingly, *RphD* had the widest range of effectiveness, and was only overcome by isolate 28.1. *RphD* has been identified in a barley land race from Tunisia (viz., Tunisian 39) and is not allelic to *Rph3*, *Rph7* or *Rph9* (Yahyaoui et al. 1988). As far as we know, this gene has not been assigned to a chromosome yet.

Rphq2 and *Rphq3* showed differential interactions in their levels of partial resistance, suggesting a "quantitative" isolate-specificity for these two QTLs. Based on the results of Qi et al. (1998b, 1999), we did not expect *Rphq4* to have an effect on the level of partial resistance at seedling stage. Indeed, the gene had no significant effect on LP of isolates 1.2.1 and 24. However, the significantly longer LP of isolate Uppsala on seedlings of L94-*Rphq4* compared to seedlings of L94 suggested an isolate-specific effect of this QTL at seedling stage (Table 4). These results were contradicted by the results obtained on the mapping population and on the NILs evaluated in the field. Isolate Uppsala was selected for the effect of *Rphq4* in seedlings, whereas later this isolate distinguished itself for having overcome *Rphq4* in seedlings and in adult plants. The isolate 17 was selected because *Rphq2* and *Rphq3* did not have a significant effect compared to L94 and the isolate 26 because only *Rphq3* had a significant effect. But, in the mapping population, *Rphq2* and *Rphq3* were detected against all the isolates, and *Rphq2* always explained a higher percentage of variation and always had a stronger additive effect than *Rphq3*. Apparently, four seedlings per NIL – *P. hordei* isolate combination were too few to obtain a reliable estimation of the effect of each QTL in its NIL background under greenhouse conditions. Testing the NILs under field conditions provided more reliable results since the disease build-up is polycyclic, each life cycle of the pathogen exponentially amplifying the small effect of individual QTLs.

To investigate the isolate-specificity of partial resistance further, the L94 × 'Vada' segregating population was challenged with the three *P. hordei* isolates, viz., 17, 26 and Uppsala, and data on isolates 1.2.1 and 24 were re-analysed. With the interval mapping method, three QTLs (*Rphq1*, *Rphq2*, *Rphq3*) had a significant effect to the five isolates while one QTL (*Rphq7*) had only a significant effect to isolate 24. This has already been reported by Qi et al. (1999) who claimed the isolate-specificity of *Rphq7* at seedling stage. However, by IM a LOD peak higher than 2 was consistently observed at *Rphq7* with all isolates (Fig. 2) and by rMQM *Rphq7* had a significant effect to two isolates, 24 and Uppsala (Table 5), indicating that *Rphq7* was not really isolate-specific but at most varied quantitatively in its

effect to the different isolates. With the rMQM method the LOD profiles of the five isolates were surprisingly less consistent than with the IM method (Fig. 2). The most surprising were the four QTLs having a significant effect by rMQM while they remained undetected by IM (*Rphq4*, *Rphq17*, *Rphq20*, *Rphq21*). It is remarkable that those four QTLs happened to be the four QTLs detected with one or two rust isolates but not with the other isolates. For the other, constant, QTLs a consistent peak was seen for all isolates with the IM and with the rMQM methods, even if *Rphq1* was not always significant with the rMQM method. At the chromosomal location of *Rphq4*, LOD peaks of 1.5 and 2.7 to isolates 17 and 26 already suggested the effect of this QTL by IM. However, this was not the case for *Rphq17*, *Rphq20* and *Rphq21*. On chromosome 3H, *Rphq17* has previously been reported in a linkage disequilibrium study (RLP4, Kraakman et al. 2006) and in the OWB population (Marcel et al. 2007). In the linkage disequilibrium, OWB and L94 × 'Vada' mapping populations the peak marker of the LP prolonging gene mapped to the same position (within 1 cM) of the barley consensus map of Marcel et al. (2007). The linkage disequilibrium population was inoculated with isolate IVP2000 and the OWB population with isolate 1.2.1. Our data suggest that *Rphq17* is not effective to isolate 1.2.1, implying that in different cultivars different alleles are involved in the level of resistance. *Rphq20* and *Rphq21* represent new loci for partial resistance to barley leaf rust. *Rphq20* was co-locating, but in repulsion phase, with the powdery mildew resistance gene *mlo* (Qi et al. 1998a) and with the quantitative resistance to scald *Rrsq2* (Shtaya et al. 2006b), which are both segregating in L94 × 'Vada'. The increase in number of detected QTLs from the IM to the rMQM method can be due to an increase in power or to an increase in the type I error rate (i.e. a QTL is indicated at a location where actually no QTL is present) (Jansen 1994). Simulation studies demonstrated that the chance of a type I or type II error (i.e. a QTL is not detected) is higher in interval mapping than it is in simultaneous mapping of multiple QTLs (Jansen et al. 1994). The use of marker cofactors in MQM mapping strongly reduces the genetic variation induced by nearby QTLs (Jansen 1994). It is less clear however, how a marker cofactor determined on one chromosome will affect the detection power of QTLs on other chromosomes. But one can imagine that cofactor analysis will be more powerful to detect unlinked QTLs with epistatic effect since the variation of possible QTL on other chromosomes is regressed on marker cofactors. We confirmed this hypothesis by showing that *Rphq17* and *Rphq20* had synergistic interactions with the most consistent QTLs *Rphq2*, *Rphq3* and *Rphq4*, and between each other (Fig. 3). A synergistic effect means that the combined effect of the administration of two compounds may be greater than the sum of the two effects. The observed synergistic interactions of *Rphq17* and *Rphq20* with other QTLs validate the effect of those loci on the level of partial resistance, and implies that: 1) MQM mapping is indeed more powerful to detect QTLs than interval mapping, 2) that QTLs can show isolate-specificity at seedling stage and 3) that epistasis between QTLs probably plays an important role in partial resistance.

The results obtained in the greenhouse on seedlings of the RIL population and the results obtained in the field on adult plants of the NILs were generally in good agreement with previous reports (Qi et al. 1998b; Niks et al. 2000a). *Rphq2*, having a strong effect in seedlings (Fig. 2) and almost no effect in adult plants (Fig. 4), was clearly plant-stage dependent, while *Rphq3* was always effective and not plant stage dependent. The isolate-specificity of *Rphq4* on seedlings was also confirmed on adult plants in the field experiments. *Rphq4* had an effect on seedlings against only two isolates 17 and 26, to which *Rphq4* explained 18% and 10% of the total variation explained by the significant QTLs. On NILs in the field, the effect of *Rphq4* on the level of partial resistance was always high against isolates 17 and 26, moderate against isolate 1.2.1 and absent against isolate Uppsala.

The differences found in the effect of *Rphq4* between isolates are consistent with the minor-gene-for-minor-gene model of Parlevliet and Zadoks (1977), which suggests specific interactions between QTLs for partial resistance and *Puccinia hordei* isolates. In their integrated concept of disease resistance, Parlevliet and Zadoks (1977) considered that all genes for true resistance in the host population, whether they are major or minor genes, are considered to interact in a gene-for-gene way with genes for (a)virulence, either major or minor, in the pathogen population. The isolate-specificity of *Rphq4* is consistent with the idea that QTLs for partial resistance encode proteins that act as pathogenicity targets interacting with specific elicitor proteins from the pathogen. So, while partial resistance appears isolate-non-specific on the whole, the individual QTLs composing this resistance appear to interact in a gene-for-gene manner and could be overcome by the pathogen.

Polygenic, quantitative resistance is believed highly durable. However, there is hardly any experience with large-scale usage of quantitative resistance over a long period to confirm this statement (Lindhout 2002). If QTLs for partial resistance function in a gene-for-gene manner, as non-durable genes associated with a hypersensitive response do, the durability of partial resistance becomes questionable. However, the fact that *Rphq2* and *Rphq3* were effective against isolate Uppsala while *Rphq4* was overcome by the same isolate suggests that, if the pathogen succeeds in breaking down the resistance of a QTL, this success does not necessarily imply that it will succeed in neutralising the effects of the other QTLs. This is of importance for the durability of partial, polygenic resistance because when one or several genes for quantitative resistance become ineffective against the pathogen, a subset of genes conferring a certain degree of quantitative resistance will always remain. So, the sum of the actions of each individual QTL against a pathogen race or isolate forms a “multiple-gene barrier” very difficult for the pathogen to overcome. A multiple-gene barrier is composed of minor genes interacting in a minor-gene-for-minor-gene way with the pathogen. If individual minor resistance genes can be overcome by the pathogen, the remaining genes that form the multiple-gene barrier will confer a sufficient level of resistance to reduce the rate by which the new virulence factors will spread through cultivated areas.

In summary, the results revealed several important features of quantitative resistance against *P. hordei* in barley. Some of the genes underlying QTL may be involved in defence responses to particular isolates of *P. hordei* and others may be more commonly involved in defence responses to a broader range of isolates. Because partial resistance is governed by several genes with different degrees of isolate-specificity, the minor-gene-for-minor-gene interaction model seems more stable than the monogenic gene-for-gene interaction model. The apparent synergistic interactions between QTLs is also intriguing in respect of the underlying resistance mechanisms. The knowledge of gene sequences underlying the QTLs would offer prospects for understanding their potential structural relationship and to gain insight into the basis of this form of durable disease resistance.

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CHAPTER 6

General Discussion

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Experimental field in 2005

General discussion

Introduction

In the past years, many genes that control Mendelian traits, or major genes, have been cloned, but the genes underlying quantitative traits (i.e. minor genes) are still hardly understood at the DNA level. The tendency of genetic background to influence the expression of phenotypes of most if not all Mendelian traits suggests that many are genetically complex. The genetic dissection of such complex traits and the identification of underlying minor genes at the DNA level has great potential in revealing the function of genes that remain uncharacterised so far.

Large-scale genome sequencing projects are generating a wealth of genomic information that require the automated prediction and annotation of genes and gene functions. A large proportion of predicted genes cannot be functionally annotated with the methods available (genes with an "unknown" function) and a proportion of the predicted genes and annotated functions are likely to be erroneous. To unravel the true biological function(s) of a gene, direct experimental procedures are required through forward or reverse genetic approaches. While forward genetics seeks to find the genetic basis of a phenotype or trait, reverse genetics seeks at identifying the function of a cloned segment of DNA or a protein sequence. Forward genetics aim at identifying the genes encoding for a determined function, while reverse genetics aim at identifying the function of a cloned segment of DNA or a protein sequence. In this thesis, we followed map-based cloning and candidate gene approaches (forward genetics) to get more insight into the nature and function of minor genes for basal resistance to *Puccinia hordei* in barley.

In the previous chapters of this thesis, we presented two barley consensus maps with 3,258 molecular markers and 775 microsatellite markers, respectively. We used the first map to compare the position of QTLs for partial resistance to leaf rust across barley populations and to identify candidate genes to explain those QTLs. The second map constitutes a great resource of mapped microsatellite markers for barley breeders and scientists, and proved useful in the identification of markers closely linked to our target loci. We also developed two sets of near-isogenic lines (NILs) containing individual or combined alleles of QTLs in susceptible and in resistant genetic backgrounds. Those NILs were instrumental in the initiation of a map-based cloning approach and in the high-resolution mapping of one minor gene, *Rphq2*. In the present chapter, we will discuss the methodology followed to construct high-density consensus maps, and the function of candidate genes identified as well as the possible nature of QTLs for partial resistance.

The construction of high-density consensus linkage maps

To date, most genetic analyses to identify genes responsible for polymorphic traits, whether they have a Mendelian or a quantitative, polygenic inheritance, in barley mapping populations or in barley linkage disequilibrium panels, have been performed with RFLP, SSR and AFLP molecular markers. Thus, the knowledge accumulated during the past 15 years through phenotyping and genotyping a number of mapping populations relies on the availability of genetic linkage maps giving the precise position of those molecular markers. GrainGenes (http://wheat.pw.usda.gov) is an international database for genetic and genomic information about Triticeae and serves as both a data repository and information hub (Carollo et al. 2005). For barley, no less than 51 linkage maps, 5 consensus linkage maps, 242 genes and more than 400 QTLs have been deposited or recorded in GrainGenes. Each mapping population has been genotyped with various sets and types of molecular markers and in each of those populations only a limited set of genes and QTLs are segregating. Then, one of the greatest challenges is the integration of these different maps, often constructed by different research groups using different techniques and different mapping populations, to produce a unified picture of the barley genome. In the past, two consensus maps based on RFLP markers (Langridge et al. 1995; Qi et al. 1996), containing 587 and 880 markers, respectively, and one consensus map combining 700 RFLP, AFLP and SSR markers (Karakousis et al. 2003) have been constructed for barley. More recently, Diab (2006) merged the previously constructed consensus maps to obtain a higher-density of markers.

Major problems encountered when constructing a consensus map with large and multiple data sets are the often limited number of loci that have been mapped in common between the individual data sets, the nomenclature of loci that may differ between data sets, the accuracy of individual data sets determined by the population type, population size and data quality, and the excessive computation time that is required for the huge data sets that are subject of the integration effort. A data set of several thousands of markers cannot be analysed with an average mapping software. In Chapters 2 and 3 of the present thesis, we used microsatellites to integrate the maps of several barley mapping populations and to construct high-density consensus maps. Microsatellite markers are among the most popular molecular markers that can be developed from ESTs. Their popularity is largely due to the fact that they require small amounts of sample DNA, are easy to detect by PCR, are amenable to high-throughput analysis, co-dominantly inherited, multi-allelic, highly informative, abundant in genomes, and highly reproducible between labs (Powell et al. 1996; Gupta and Varshney 2000). We proposed a 7 steps method to construct a consensus map gathering several thousands of genetic markers (Fig. 1). The proposed method relies on improved map construction programmes (RECORD: Van Os et al. 2005a; JoinMap® 4.0: http://www.kyazma.nl) and on the “neighbours” map approach described by Cone et al. (2002).

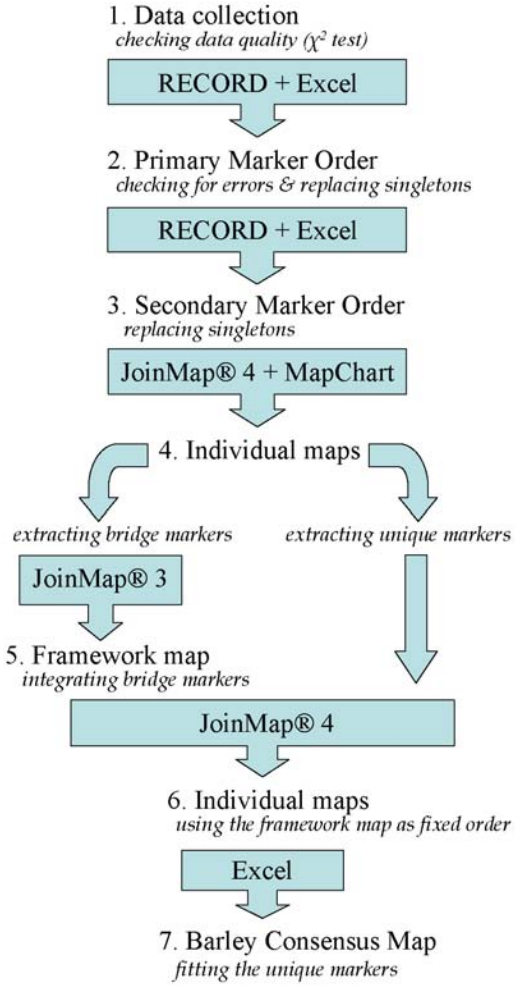


Figure 1. Flow chart of the method used to construct the consensus maps of barley. The *numbers* represent the different steps and the *grey arrows* the computer programs used for calculations.

Step 1. A chi-square test is run on the individual data sets to identify the regions associated with distorted segregation and to remove isolated markers showing significant skewness.

Step 2. The markers of each data set are ordered with the programme RECORD and sorted by graphical genotyping in Microsoft® Office Excel to remove singletons (i.e. conflicting data points) and other potential errors.

Step 3. The *step 2* is repeated to ensure that a minimum number of errors remain in the data sets before constructing the map.

Step4. The individual maps of each data set are calculated with the (Monte Carlo) maximum likelihood mapping option of JoinMap® 4.0.

Step5. The individual maps are aligned in MapChart© 2.2 to identify bridge markers in common between at least two linkage maps. The bridge markers are extracted from the data

sets and used to calculate a framework map using the integrative function of the regression mapping method available in JoinMap® 4.0.

Step 6. The individual maps of each data set are recalculated with the (Monte Carlo) maximum likelihood mapping method by adding the order of the framework markers as a "fixed order file" into JoinMap® 4.0.

Step 7. The framework map served as fixed backbone onto which the unique loci of each newly calculated individual map are added following the "neighbours" map approach.

The construction of (ultra) high-density consensus maps requires adequate tools and techniques to reduce the computation times while keeping the accuracy of the constructed maps as high as possible. For the needs of the potato genome sequencing consortium, which aims at elucidating the complete DNA sequence of the potato genome, Van Os et al. (2006) constructed an ultra-dense genetic linkage map with nearly 10,000 AFLP loci. To reach this objective, new tools were developed (Van Os et al. 2005a, 2005b). The first one, SMOOTH, identifies and removes singletons from genetic mapping data sets. The second one, RECORD, optimises the ordering of a large number of loci on genetic linkage maps in a limited amount of computation time. RECORD employs a marker-ordering algorithm based on the minimisation of the total number of recombination events in any given marker order. The removal of singletons by graphical genotyping (SMOOTH can only handle backross and F_2 populations) together with the use of RECORD allowed us to efficiently prepare our data sets before map integration in JoinMap®, which appears to be the only software option available that allows to integrate datasets of populations derived from crosses between different pairs of parents (Stam 1993). It has already been reported that the simultaneous use of RECORD and JoinMap® 3.0 improves the construction of genetic linkage maps (Wenzl et al. 2006; Vromans et al. 2007). The release of JoinMap® 4.0 in August 2006 offered another tool to handle large amounts of data. The (Monte Carlo) maximum likelihood algorithm implemented in JoinMap® 4.0 allowed a very fast computation of our data sets.

Recently, Wenzl et al. (2006) proposed an alternative procedure to construct a high-density consensus map of barley, comprising 2,935 loci, using a combination of RECORD, JoinMap® 3.0 and several purpose-built perl scripts. The main steps proposed for map construction are essentially the same as presented in our study: preparation of quality-filterered datasets, construction of component maps, construction of a skeleton map and fitting remaining loci on the rigid skeleton map. They obtained a map that integrates 850 "traditional" loci, mostly RFLP and SSR markers also present on our consensus map, with 2,085 DArT loci from ten different mapping populations. This effort demonstrated the power of DArT assays and provides a first bridge between the existing genetic knowledge of barley and DArT markers produced by hybridisation-based technologies. An alignment of the

markers in common between the consensus map presented in Chapter 2 of this thesis and the consensus map released by Wenzl et al. (2006) is presented in Figure 2. Map length and marker order are influenced by many factors, including the frequency of double recombinants, the occurrence of errors in scoring and data input, and changes in local or general recombination behaviours. The degree of map expansion tended to be slightly higher in the consensus map of Wenzl et al. (2006). The marker order between the two maps is as a whole consistent with most of the ambiguities concentrated around the centromeres. The most conflicting marker on chromosome 6H was ksuA3B. The position of this marker on independent linkage maps (Kleinhofs et al. 1993) suggests that the position on the consensus map of Marcel et al. (2007) is the most reliable one.

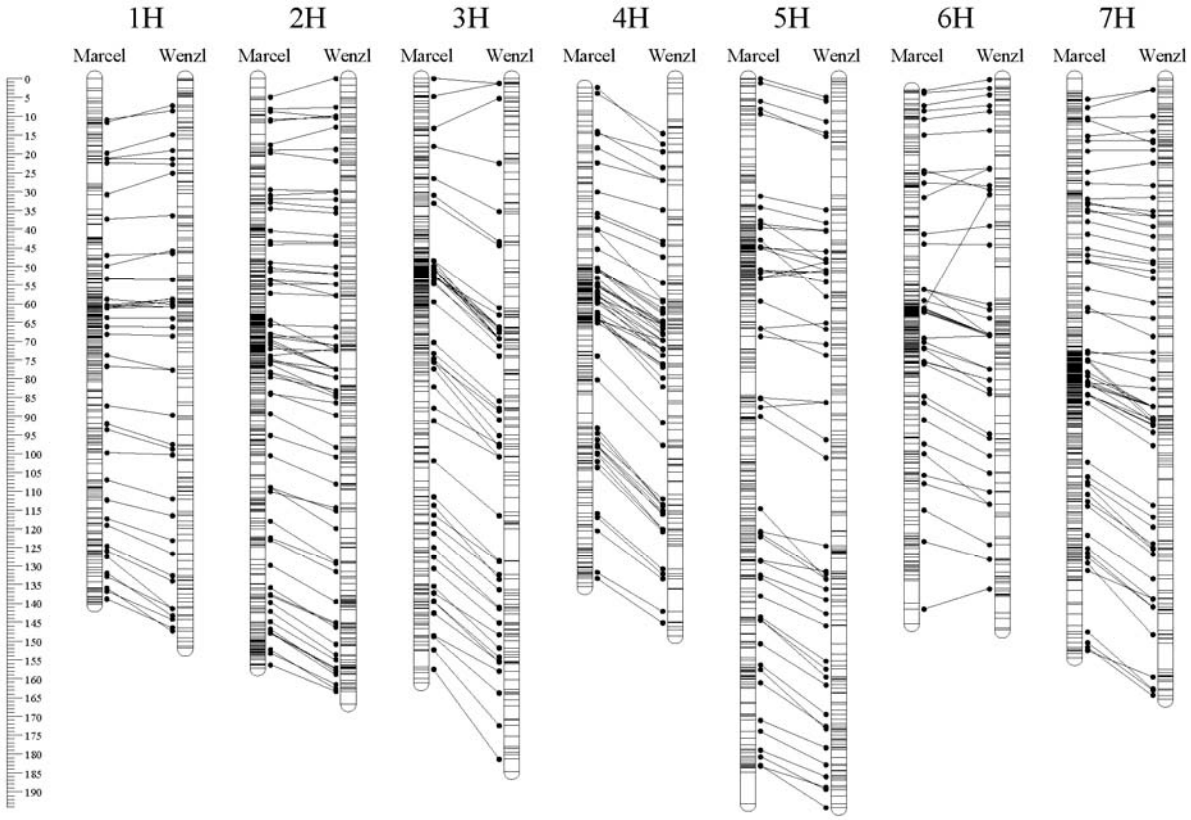


Figure 2. Alignment of two high-density barley consensus maps. Comparison of locus positions shared between the consensus map of Marcel et al. (2007) presented in Chapter 2 of this thesis, containing a majority of AFLP, RFLP and SSR loci, and the consensus map of Wenzl et al. (2006), containing a majority of DArT loci and 850 other loci. The position of each shared locus between the two maps is highlighted by a *pair of dots connected by a line*.

Co-localisation of known major genes and mapped QTLs in barley

The first aim of the high-density consensus map of barley presented in this thesis was to offer a comparative tool between different barley genetic maps in which genes for resistance to

P. hordei have already been placed. Previously, Qi et al. (1998b) compared the position of known major genes (*Rph* genes) and partial resistance QTLs (*Rphq* loci) to *P. hordei* using a barley integrated RFLP map. They concluded that there was no indication of shared map positions between eleven major resistance genes and six QTLs for partial resistance identified in the L94 × 'Vada' mapping population. Since then, two additional *Rph* loci have been mapped, i.e. *Rph16.ae* and *Rph19.ah* (Ivandic et al. 1998; Park and Karakousis 2002), and a literature survey indicated that QTLs identified in thirteen additional studies could also be placed on the present barley consensus map (Spaner et al. 1998; Qi et al. 1999; Kicherer et al. 2000; Qi et al. 2000; Backes et al. 2003; von Korff et al. 2005; Jafary et al. 2006a; Jafary et al. 2006b; Kraakman et al. 2006; Rossi et al. 2006; Marcel et al. 2007; Jafary et al. unpublished; Marcel et al. unpublished). The nomenclature of the *Rph* loci follows the recommendations of Franckowiak et al. (1997). We extended the analysis of Qi et al. (1998b) by placing most loci for resistance to barley leaf rust identified so far on the new consensus map (Fig. 3). The most striking conclusion of this exercise concerns the abundance of QTLs on the genome of barley. This evidence was already pinpointed by several authors (Qi et al. 2000; Jafary et al. 2006a; Marcel et al. 2007), who have shown, one after the other, that each parental barley combination segregates for different sets of QTLs, with only few QTLs shared by any pair of cultivars. Among the 29 barley genomic regions conferring quantitative resistance to leaf rust, only 6 were detected in more than two different barley cultivars, viz. three on the long arm of chromosome 2H and one each in the centromeric region of chromosomes 3H, 6H and 7H (Fig. 3). The paucity of QTLs segregating in common indicates that the frequency of the resistance allele for partial resistance genes is either very low or very high. Nevertheless, we can note that the four QTLs detected by linkage disequilibrium in a panel of 148 barley cultivars were all supported by the overlapping position of QTLs detected in individual mapping populations (Kraakman et al. 2006). Other QTLs were confirmed across mapping populations that share an identical parental line, viz. L94 × 'Vada' and SusPtrit × 'Vada' (Qi et al. 1998b; Jafary et al. 2006a), and L94 × (L94 × Cebada Capa) and SusPtrit × Cebada Capa (Qi et al. 2000; Jafary et al. 2006b). Finally, the performance of NILs evaluated in Chapters 4 and 5 of this thesis also confirmed the effect of three QTLs.

About half of the major *Rph* resistance genes shared map position with a QTL. Some QTL studies have been performed in field experiments under natural leaf rust infection (Spaner et al. 1998; von Korff et al. 2005; Rossi et al. 2006). If the natural inoculum consisted of a mix of virulent and avirulent rust isolates to a *Rph* gene segregating in the investigated population, then this *Rph* gene would appear as a "QTL" in the study. Anyhow, considering that the confidence intervals of QTLs cover more than 35% of the consensus map and that genes are more likely to be clustered in gene-rich regions, there is no reason to think that loci for qualitative and loci for quantitative resistances to leaf rust in barley are associated and evolutionary related to each other.

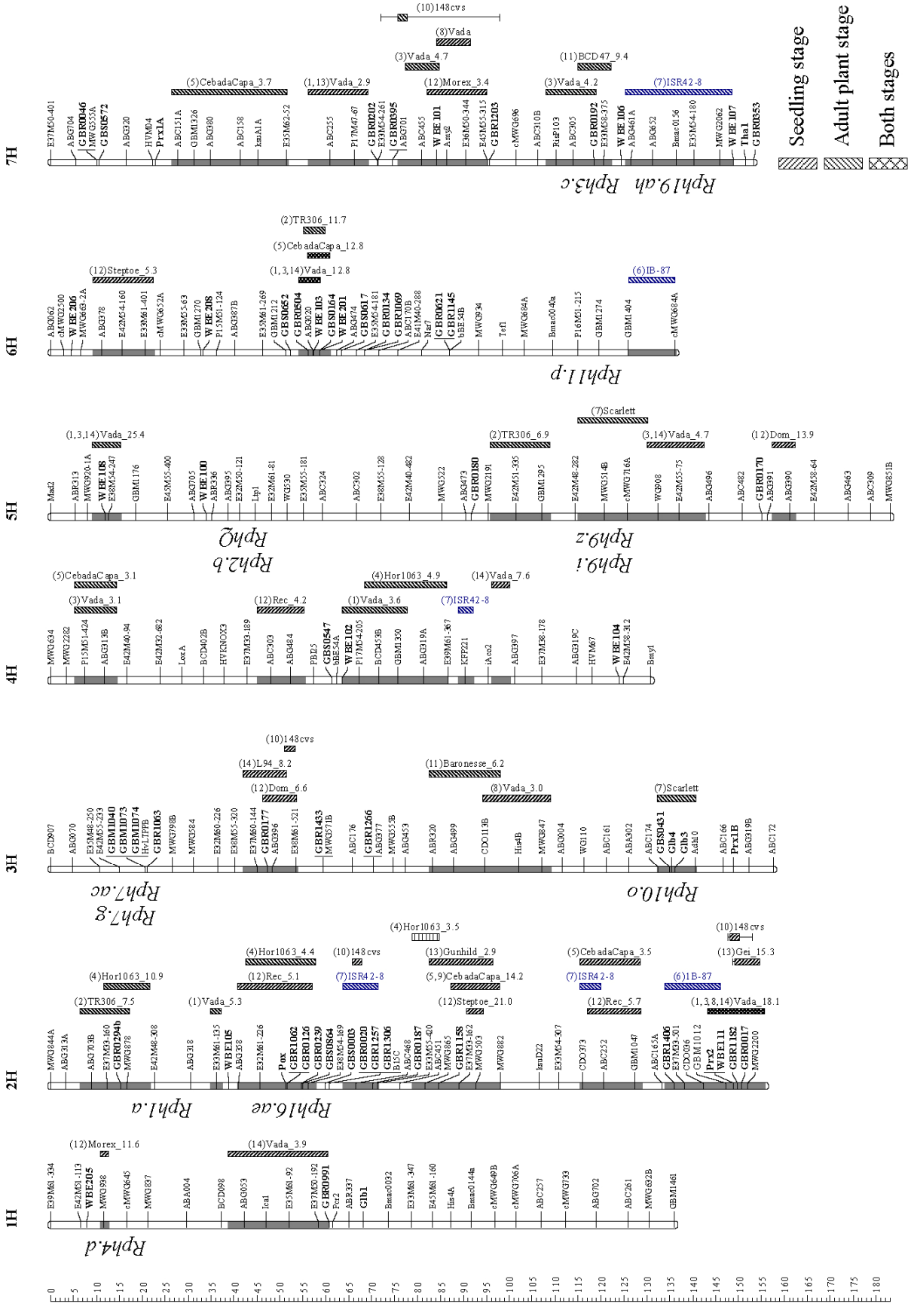

7H
(10)148cvs
(8)Vada
(3)Vada_4.7
(11)BCD47_9.4
(5)CebadaCapa_3.7
(1,13)Vada_2.9
(12)Morex_3.4
(3)Vada_4.2
(7)ISR42-8
Seedling stage
Adult plant stage
Both stages
Rph19.ah Rph3.c
6H
(2)TR306_11.7
(5)CebadaCapa_12.8
(12)Steptoe_5.3
(1,3,14)Vada_12.8
(6)IB-87
Rph11.p
5H
(7)Scarlett
(1,3,14)Vada_25.4
(2)TR306_6.9
(3,14)Vada_4.7
(12)Dom_13.9
Rph2.b
RphQ
Rph9.i
Rph9.z
4H
(5)CebadaCapa_3.1
(4)Hor1063_4.9
(14)Vada_7.6
(3)Vada_3.1
(12)Rec_4.2
(1)Vada_3.6
(7)ISR42-8
3H
(10)148cvs
(14)L94_8.2
(11)Baronesse_6.2
(12)Dom_6.6
(8)Vada_3.0
(7)Scarlett
Rph7.g
Rph7.ac
Rph10.o
(4)Hor1063_3.5
(10)148cvs
(4)Hor1063_4.4
(10)148cvs
(13)Gunhild_2.9
(5)CebadaCapa_3.5
(13)Gei_15.3
(4)Hor1063_10.9
(12)Rec_5.1
(7)ISR42-8
(5,9)CebadaCapa_14.2
(7)ISR42-8
(6)1B-87
(2)TR306_7.5
(1)Vada_5.3
(12)Steptoe_21.0
(12)Rec_5.7
(1,3,8,14)Vada_18.1
2H
Rph1.a
Rph16.ae
1H
(12)Morex_11.6
(14)Vada_3.9
Rph4.d
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180

Figure 3. (Previous page). BIN map extracted from the high-density consensus map of barley displaying the position of *Rph* loci for hypersensitivity resistance *on the left side of chromosome bars* and the position of *Rphq* loci for partial resistance *on the right side of chromosome bars*. The *name on QTL bars* has three components: the reference number, the name of the cultivar that gave the resistance allele and the highest LOD value recorded for the QTL. The references are (1) Qi et al. 1998b; (2) Spaner et al. 1998, (3) Qi et al. 1999; (4) Kicherer et al. 2000 ; (5) Qi et al. 2000 ; (6) Backes et al. 2003; (7) von Korff et al. 2005 ; (8) Jafary et al. 2006a ; (9) Jafary et al. 2006b; (10) Kraakman et al. 2006; (11) Rossi et al. 2006; (12) Marcel et al. 2007; (13) Jafary et al. unpublished; (14) Marcel et al. unpublished. The QTLs discovered by association mapping are represented by a *line covering the area associated with resistance* and by a *box of 2 cM around the most associated marker*. *Grey coloration within chromosome bars* represent the area covered by the confidence intervals of QTLs. The 63 loci in *bold* are DGH-based markers. The *ruler on the left end side* of the figure indicates the distance in centiMorgans (according to Kosambi) from the top of each chromosome.

In Chapter 4 of this thesis, we focused on the high-resolution mapping of quantitative resistance *Rphq2* located near the telomere of chromosome 2HL of 'Vada'. The *Rphq2* locus has been transferred to European cultivars from the 'botanical' barley line *Hordeum laevigatum* together with the tightly linked race-specific resistance genes *MlLa* and *Rdg1a* for resistance to powdery mildew and to leaf stripe, respectively (Giese et al. 1993; Arru et al. 2002). In this study, *MlLa* was mapped distally from *Rphq2* excluding the possibility that the same gene is responsible for quantitative resistance to leaf rust and qualitative resistance to powdery mildew. Arru et al. (2002) mapped *Rdg1a* about 10 cM proximal from the peak marker of *Rphq2*. In a leaf stripe disease test, L94-*Rphq2* was also as susceptible as the susceptible parent L94 (N. Pecchioni, personal communication) excluding as well the possibility that *Rphq2* and *Rdg1a* are the same gene. Those observations support the idea that partial/quantitative resistance, based on a pre-haustorial mechanism with the formation of papillae, and qualitative resistance, based on a post-haustorial mechanism with hypersensitivity, are distinct and not associated with each other.

Co-localisation of defence gene homologues with mapped QTLs in barley

The level of partial resistance is related to the failure rate of haustorium formation (Niks 1982, 1983a, 1986). Failed haustorium formation is associated with papilla formation. The contact between the haustorial mother cell with the cell wall, and possibly the contact of the penetration peg with the plant cell membrane is the critical stage in which the failure or success of the haustorium formation seems to be determined. A very susceptible line (L94) is much less able to stop haustorium formation by *Puccinia hordei* than a cultivar with high level of partial resistance ('Vada'). Still, line L94 has a substantial level of prehaustorial resistance to the two inappropriate rusts *P. tritici* and *P. hordei-murini* (Niks 1983a, 1983b; Hoogkamp et al. 1998). Therefore, L94 is perfectly able to block haustorium formation by

rusts and to form papillae. This suggests that the "defence machinery" in L94 is as good as in Vada, but the difference between 'Vada' and L94 is how easily this defence is activated or suppressed. Considering the above observations, the functions of the candidate genes identified in Chapter 2 of this thesis, receptor-like kinase (RLK), *WIR1* homologues, peroxidase, superoxide dismutase, and thaumatin, in the defence response underlying partial resistance are discussed.

Table 1. Comparison of the number of members within several multigene families known to be involved in the defence response to pathogens in *Arabidopsis* and in rice

Gene Family	Function	*Arabidopsis*	Rice	Reference
Detection				
NBS-LRR	Resistance protein	149	< 500	Meyers et al. 2003 Monosi et al. 2004
RLK/Pelle	Resistance protein	610	< 1132	Shiu & Bleecker 2003 Shiu et al. 2004
RLK-LRR[1]	Resistance protein	240	384	Shiu & Bleecker 2001 Morillo & Tax 2006
WAK/WAKL[1]	Resistance protein	26	125	Verica et al. 2003 Zhang et al. 2005
Signalling				
PR-9 Peroxidase	Multifunctional	73	138	Bakalovic et al. 2006
MAPKKK	Signalling molecule	60	?	Nakagami et al. 2005
MAPKK	Signalling molecule	10	?	Nakagami et al. 2005
MAPK	Signalling molecule	20	17	Nakagami et al. 2005 Reyna & Yang 2006
WRKY	Transcription factor	> 70	> 100	Dong et al. 2003 Zhang et al. 2005
AP2/ERF[2]	Transcription factor	?	?	Gutterson et al. 2004
bZIP	Transcription factor	82	94	Qu & Zhu 2006
Myb	Transcription factor	189	182	Qu & Zhu 2006
Response				
SOD	Antioxidant	7	?	Alscher et al. 2002
PR-5 thaumatin	Antimicrobial	21	30	Shatters Jr et al. 2006
WIR1	Unknown	0	2[3]	Mauch et al. 1998 Schaffrath et al. 2000

[1] Subfamilies of the RLK/Pelle gene family; [2] Subfamily of the AP2 gene family; [3] Probably an underestimation since the small size of those proteins hamper their detection

In *Arabidopsis*, RLKs belong to a large gene family (RLK/Pelle) with more than 610 members (Table 1) (Shiu and Bleecker 2003). More than 400 of those members have a

domain configuration resembling transmembrane receptors, implying a major contribution of this class of proteins in the perception of cell surface signals in plants. RLKs regulate a wide variety of developmental and defence-related processes. The fact that RLKs are membrane receptors associated with the recognition of bacterial or fungal pathogens (Song et al. 1995; Feuillet et al. 1997; Gómez-Gómez and Boller 2000; Sun et al. 2004) match a putative function of QTL genes in the perception of penetration attempts by the barley leaf rust fungus at the barley cell membrane. *ROF1* is a wall-associated RLK which was recently identified as a novel type of dominant disease-resistance protein conferring resistance to a broad spectrum of Fusarium races in *Arabidopsis* (Diener and Ausubel 2005). *ROF1* is member of the WAK/WAKL gene subfamily to which roles in both development and stress-response signalling have been attributed. Diener and Ausubel (2005) hypothesised that WAKs/WAKLs are involved in both abiotic and biotic stress responses through their tight association with the cell wall and possibly through the perception of the loosening of the adhesion of the plasma membrane to the cell wall. In compatible combinations, the fungus successfully induces such loosening of the attachement between plasma membrane and cell wall. It was proven earlier that the expression of cell-wall associated defences, such as extracellular hydrogen peroxide generation and callose deposition, also depends on adhesion between the plant cell wall and the plasma membrane (Mellersh and Heath 2001). As for the WAKs/WAKLs, Bull et al. (1992) speculated a function of the *WIR1* gene related to the adhesion of the membrane to the cell wall in case of pathogen attack. PWIR1 is an integral membrane protein induced in wheat by the non-host pathogen *Erysiphe graminis* f. sp. *hordei* (Bull et al. 1992). In barley, homologues of *WIR1* were also found to be induced upon inoculation with the host pathogen *Blumeria graminis* f. sp. *hordei* (Jansen et al. 2005; Zierold et al. 2005) and with the non-host pathogen *Puccinia triticina* (Neu et al. 2003). Because of their function, RLKs and *WIR1* are plausible candidates to explain QTL function for partial resistance. This hypothesis implies that the plant perceive intruding microbes like *P. hordei* and that QTL-alleles from resistant lines may encode for proteins that have a role in tightening the binding between membrane and cell wall in the plant.

Defence response (DR) genes are involved in the plant defence upon attack by microbial or insect pests. It is often assumed that DR genes like those encoding peroxidase (PR-9), superoxide dismutase (SOD) and thaumatin-like protein (PR-5) are potential candidates to explain the QTLs for quantitative resistance to plant pathogens. However, because the “defence machinery” in L94 is as good as in ‘Vada’, it is more likely that the QTLs for partial resistance to *P. hordei* play a role in the recognition or early signalling of the defence response rather than a role in the expression of the resistance. Peroxidase (H_2O_2) and superoxide dismutase ($O_2^{\cdot-}$) are reactive oxygen intermediates (ROIs). ROIs have been implicated in signal transduction as well as in the execution of defence reactions such as cell wall strengthening and a rapid hypersensitive reaction (reviewed in Hückelhoven and Kogel

2003). But the role of ROIs in the establishment and maintenance of either host cell inaccessibility or accessibility during attack by a fungal plant pathogen is not yet fully understood. Genes encoding different types of peroxidases, in different plant species, have been shown to promote susceptibility to fungal and bacterial pathogens (Kristensen et al. 2001; Coego et al. 2005; Chen et al. 2006a), suggesting that the pathogen uses early signals generated during the oxidative burst for the selective activation/suppression of host factors required for mounting a compatible interaction. One of those peroxidases, the vacuolar peroxidase *Prx7*, was implicated as a susceptibility factor in the response of barley to attack by *B. graminis* f. sp. *hordei*, enhancing successful haustorium formation (Kristensen et al. 2001). *Prx7* mapped in the same region of chromosome 2HL (Giese et al. 1993) as *Prx2* which is another peroxidase gene locus identified as a candidate to explain *Rphq2*. The mildew haustorium promoting effect of *Prx7* (Kristensen et al. 2001) qualifies peroxidase genes as candidates for QTLs for partial resistance to *P. hordei*. In this view, *Prx7* might be considered a compatibility factor that increases susceptibility of the plant. This factor would, as a ligand, be recognised by the invading pathogen, and this recognition could result in suppression of an innate defence system of the plant.

The use of synteny for the dissection of QTL regions

Cereals have widely differing genome sizes ranging from 450 Mb for rice to 5,000 Mb for barley and 16,000 Mb for hexaploid wheat (Arumuganathan and Earle 1991). This genome size variation is partly caused by differences in ploidy level, but is mainly due to differences in the amount of repetitive DNA. Nevertheless, comparative genome analyses revealed a high degree of conservation of gene content and order between cereal species that allowed the use of cereals with smaller genomes as genetic models (Moore et al. 1995; Devos and Gale 2000; Devos 2005). In particular, the recent sequencing of the rice genome (International Rice Genome Sequencing Project 2005) has provided researchers an efficient tool for cross-genome gene isolation (Xu et al. 2005).

In Chapter 4 of this thesis, we focused on the high-resolution mapping of quantitative resistance *Rphq2* located in the telomere region of chromosome 2HL of 'Vada'. By cross comparison of sequenced ESTs from barley and genes predicted from the rice sequence a number of EST-based molecular markers were placed in the vicinity of *Rphq2*. Then, the alignment of a high-resolution barley genetic map with the sequence of rice resolved the interval containing the gene responsible for *Rphq2* to a homologous rice stretch of sequence of at most 70 kb. Six predicted rice genes encoding peroxidase proteins and another predicted rice gene with high sequence similarity to a mitogen-activated protein kinase kinase kinase (MAP3K) were identified as candidates to explain *Rphq2*. Barley ESTs with high homology (10^{-129} < E value < 10^{-19}) to the candidate genes identified in rice were found by blastn

analysis, suggesting that homologues of the rice genes are present in barley. The linkage association between *Rphq2* and peroxidase genes was already observed in the DGH studies presented in Chapter 2 of this thesis. *Rphq6* and *Rphq18*, on the short arm of chromosome 2H, were also associated with stress related peroxidases and several authors reported as well such associations between peroxidase loci and genes for quantitative resistance (Faris et al. 1999; Ramalingam et al. 2003). Members of the MAP3K family are crucial for early defence signalling and cellular stress response to bacterial and fungal pathogens (Asai et al. 2002). These key signalling molecules are the first component of MAPK cascades. MAPK cascades minimally consist of a MAP3K-MAP2K-MAPK module that is associated in various ways to upstream receptors and downstream targets (reviewed in Nakagami et al. 2005; Pedley and Martin 2005). The ROI-induced activation of MAPKs has been taken as evidence that also ROI act upstream of MAPKs.

Table 2. Cloned quantitative trait loci (QTL) in plants

Species	Trait	QTL	Gene	Function	Reference
Arabidopsis	Flowering time	*EDI*	*CRY2*	Cryptochrome	El-Assal et al. 2001
Arabidopsis[1]	Gluc. structure	*GS-elong*	*MAM2*	MAM synthase	Kroymann et al. 2003
Arabidopsis	Root morphology	*BRX*	*BRX*	TF	Mouchel et al. 2004
Arabidopsis	Flowering time	*FLW1*	*FLM*	TF	Werner et al. 2005
Arabidopsis	Transpiration efficiency	*TE1*	*ERECTA*	LRR-RLK	Masle et al. 2005
Arabidopsis	Gluc. hydrolysis	*ESM1*	*MyAP*	Myrosinase AP	Zhang et al. 2006
Barley[1]	Photoperiod	*Ppd-H1*	*Ppd-H1*	Pseudo-resp.	Turner et al. 2005
Maize	Apical dominance	*Tb1*	*Tb1*	TF	Doebley et al. 1997
Maize[1]	Flowering time	*Dwarf8*	*Dwarf8*	TF	Thornsberry et al 2001
Rice	Heading time	*Hd1*	*Se1*	TF	Yano et al. 2000
Rice	Heading time	*Hd3a*	*Hd3a*	Unknown	Kojima et al. 2002
Rice	Heading time	*Hd6*	*CK2α*	Protein kinase	Takahashi et al. 2001
Rice	Heading time	*Ehd1*	*Ehd1*	B-type resp.	Doi et al. 2004
Rice	Salt tolerance	*SKC1*	*SKC1*	Sodium transp.	Ren et al. 2005
Rice	Seed dormancy	*DOG1*	*DOG1*	Unknown	Bentsink et al. 2006
Rice	Grain productivity	*Gn1a*	*CKX2*	Cytokinin o/d	Ashikari et al. 2005
Rice[1]	Grain weight/length	*GS3*	*GS3*	Unknown	Fan et al. 2006
Tomato	Fruit weight	*fw2.2*	*OFRX*	Unknown	Frary et al. 2000
Tomato	Fruit shape	*Ovate*	*Ovate*	Unknown	Liu et al. 2002
Tomato	Fruit sugar content	*Brix9-2-5*	*Lin5*	Invertase	Fridman et al. 2004

Abbreviations: Gluc., Glucosinolate ; TF, Transcription factor; LRR-RLK, leucine-rich repeat receptor-like kinase; Myrosinase AP, Myrosinase associated protein; B-type resp., B-type response regulator; Sodium transp., Sodium transporter ; Cytokinin o/d, Cytokinin oxidase/dehydrogenase; Pseudo-resp., Pseudo-response regulator

[1] Without formal complementation

Nevertheless, disruptions in the colinearity between rice and Triticeae species can severely compromise the use of synteny for the map-based isolation of genes. The degree of gene order conservation may vary, depending on the region, and is unlikely to be perfect (Rostoks et al. 2005a). This is mostly due to the presence of multigene families and to the occurrence of chromosomal and genic duplications (Devos and Gale 2000). Plant gene families are very unequal within and between plant species in terms of number of members and of evolution dynamic (Table 1). For example, resistance gene homologues are known to undergo rapid and dramatic rearrangements between related species (Leister et al. 1998). Such disruptions in colinearity between resistance gene regions has often prevented the straightforward identification of a candidate gene by proxy for large cereal genomes, such as wheat (Yahiaoui et al. 2004) and barley (Brunner et al. 2003; Perovic et al. 2004). Notably, extensive efforts to clone the barley stem rust resistance gene *Rpg1* by synteny with rice provided excellent flanking markers but failed to yield the gene because it does not seem to exist in rice (Brueggemann et al. 2002). The rice genome is indeed a good source of markers to saturate barley or wheat genomic regions but seems to be more promising for the isolation of genes that are evolutionarily more conserved (Keller et al. 2005). *Brachypodium distachyon* has recently been proposed as a new model for grass functional genomics, because it is self-fertile, has a short life cycle (less than 4 months), a small stature, simple growth requirements, a small genome size (~355 Mb) and diploid accessions are available (Draper et al. 2001). The phylogenetic position of *B. distachyon* between rice and Triticeae crops makes this species a promising alternative to rice for comparative genomics and gene discovery in its larger genome relatives (Draper et al. 2001; Hasterok et al. 2006). However, although genetic and genomic tools are developing rapidly for *B. distachyon*, the usefulness of that species for cross-genome gene isolation still remains to be practically demonstrated.

Nature of genes responsible for quantitative traits

Plants use a variety of strategies to defend themselves against microbial attacks. One important defence mechanism is the recognition by plant resistance (*R*) genes of the presence of specific pathogen effectors or *Avr* gene products that leads to a hypersensitive response. Most of the *R* genes for hypersensitive resistance that have been isolated belong to the same gene family, the nucleotide binding site leucine-rich repeat (NBS-LRR). In the barley – barley leaf rust system, more than 31 major genes (*Rph* genes) conferring hypersensitive resistance have been identified (Franckowiak et al. 1997). These *Rph* genes have been mapped to 10 distinct loci (Fig. 3) but none of them has yet been isolated. The research presented in this thesis focused on another plant defence system based on minor genes or QTLs that act in a pre-haustorial, non-hypersensitive manner. One strong conclusion of our work is that there is a great diversity of QTLs for partial resistance to leaf rust in the barley genome, with 52

QTLs, delimiting 29 genomic regions, that have been identified in the 14 studies reviewed (Fig. 3). It seems therefore natural to wonder whether all the minor genes explaining those QTLs belong to a unique gene family as most *R*-genes do, or belong to many different gene families implicated in the defence response. If minor genes for partial resistance can be explained by only one gene family, members of the RLK/Pelle family are attractive candidates because a multitude of them are found in plant genomes (Table 1) (Morillo and Tax 2006), and because they have already been identified as MAMP-receptors (Nürnberger et al. 2004) and as candidate genes in the present study (Chapter 2). However, the large number of candidate genes identified does not clearly implicate any single gene family in particular. We therefore favour at the moment the alternative hypothesis, that QTLs for disease resistance are conditioned by genes of different nature.

Proof of gene discovery has been obtained for at least 20 QTLs in plants, mostly in rice and *Arabidopsis* (Table 2). Among them, several are transcription factors (Table 2). Transcription factors are a group of proteins that control cellular processes by regulating the expression of downstream target genes (Qu and Zhu 2006). Four families of transcription factors have been implicated in the defence response of plants to pathogens (Table 1) (Gutterson and Reuber 2004; Thurow et al. 2005; Zhang and Wang 2005; Chen et al. 2006b). Also, several of the minor genes isolated are genes of unknown function (Table 2), suggesting that cloning QTL genes is promising for the functional understanding of plant genomes.

Partial resistance is usually considered as a durable type of disease resistance, to which pathogens not easily adapt. As QTLs can confer a high level of resistance by their accumulated effects, the adaptation of a pathogen to render each QTL resistance-allele ineffective is theoretically more complex than to render just one gene ineffective in case of monogenic resistance (Lindhout 2002), especially when each QTL-gene encodes a different (type of) gene product. In Chapter 5, we presented evidence that individual QTLs can be effective to one isolate but ineffective to another isolate of *P. hordei*. This is consistent with the minor-gene-for-minor-gene hypothesis proposed by Parleveliet and Zadoks (1977). In the field experiments, the fact that only one QTL became ineffective to *P. hordei* Uppsala while the other QTLs remained effective also indicated that the pathogen cannot overcome all QTLs at once. The isolate-specificity of QTLs suggests a certain plasticity and diversity of the underlying supposed partner genes, i.e. the targeted plant genes and the corresponding pathogen effectors. Rubiales and Niks (2000) suggested that durability may depend as well on a combination of genes affecting different mechanisms of resistance. If the hypothesis that QTLs for partial resistance are explained by multiple genes of different nature can be proven right, this could be another factor explaining the durability of this type of resistance. As highlighted by the results presented in Chapter 5, epistasis seems also to be an important component of the genetic architecture of partial resistance. Moore et al. (2005) explained that epistasis creates dependencies among the genes in a network providing a sort of genetic

buffering against the effects of mutations. This buffering helps to stabilise complex traits like partial resistance so improving its durability.

Concluding remarks

Thanks to the tools developed, we learned that QTLs for partial resistance to leaf rust are very abundant in the barley genome with different sets of QTLs segregating in each mapping population tested. Many QTLs act in epistasis and individual QTLs can have a specific effect to only some isolates of a same fungal species. This was however not the case for *Rphq2* that does not seem to depend on such epistatic interactions, having a conserved effect in different genetic backgrounds, and was effective to all isolates tested. This gene is therefore a good candidate for cloning and verification by complementation in the future. We also learned that *Rphq2* is explained by a single gene with an incompletely dominant effect. And we confirmed that *Rphq2* has a predominantly non-hypersensitive resistance mechanism that is likely to be involved in the basal resistance of barley to leaf rust. We hope that this work will facilitate the map-based isolation of a plant gene for partial resistance.

References

Alonso-Blanco C, Koornneef M (2000) Naturally occurring variation in *Arabidopsis*: an underexploited resource for plant genetics. TRENDS Plant Sci **5**: 22–29

Alscher RG, Erturk N, Heath LS (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J Exp Bot **53**: 1331–1341

Andersen JR, Lübberstedt T (2003) Functional markers in plants. TRENDS Plant Sci **8**: 554–560

Andersen-Nissen E, Smith KD, Strobe KL, Barrett SL, Cookson BT, Logan SM, Aderem A (2005) Evasion of Toll-like receptor 5 by flagellated bacteria. Proc Natl Acad Sci USA **102**: 9247–9252

Anderson JA, Churchill GA, Autrique JE, Tanksley SD, Sorrels ME (1993) Optimizing parental selection for genetic linkage maps. Genome **36**: 181–186

Anikster Y, Wahl I (1979) Coevolution of the rust fungi on Gramineae and Liliaceae and their hosts. Ann Rev Phytopathol **17**: 367–403

Arru L, Niks RE, Lindhout P, Valé G, Francia E, Pecchioni N (2002) Genomic regions determining resistance to leaf stripe (*Pyrenophora graminea*) in barley. Genome **45**: 460–466

Arru L, Francia E, Pecchioni N (2003) Isolate-specific QTLs of resistance to leaf stripe (*Pyrenophora graminea*) in the 'Steptoe' x 'Morex' spring barley cross. Theor Appl Genet **106**: 668–675

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Report **9**: 208–218

Asai T, Tena G, Plotnikova J, Willmann MR, Chiu W-L, Gomez-Gomez L, Boller T, Ausubel FM, Sheen J (2002) MAP kinase signalling cascade in *Arabidopsis* innate imunity. Nature **415**: 977–983

Ashikari M, Sakakibara H, Lin S, Yamamoto T, Takashi T, Nishimura A, Angeles ER, Qian Q, Kitano H, Matsuoka M (2005) Cytokinin oxidase regulates rice grain production. Science **309**: 741–745

Atienza SG, Jafary H, Niks RE (2004) Accumulation of genes for susceptibility to rust fungi for which barley is nearly a nonhost results in two barley lines with extreme multiple susceptibility. Planta **220**: 71–79

Backes G, Madsen LH, Jaiser H, Stougaard J, Herz M, Mohler V, Jahoor A (2003) Localisation of genes for resistance against *Blumeria graminis* f.sp. *hordei* and *Puccinia graminis* in a cross between a barley cultivar and a wild barley (*Hordeum vulgare* ssp. *spontaneum*) line. Theor Appl Genet **106**: 353–362

Bai Y, Huang C-C, van der Hulst R, Meijer-Dekens F, Bonnema G, Lindhout P (2003) QTLs for tomato powdery mildew resistance (*Oidium lycopersici*) in *Lycopersicon parviflorum* G1.1601 co-localize with two qualitative powdery mildew resistance genes. Mol Plant-Microbe Interact **16**: 169–176

Bai Y, Feng X, van der Hulst R, Lindhout P (2004) A set of simple PCR markers converted from sequence specific RFLP markers on tomato chromosomes 9 to 12. Mol Breeding **13**: 281–287

Bakalovic N, Passardi F, Ionnidis V, Cosio C, Penel C, Falquet L, Dunand C (2006) PeroxiBase: A class III plant peroxidase database. Phytochemistry **67**: 534–539

Becker J, Heun M (1995) Barley microsatellites: allele variation and mapping. Plant Mol Biol **27**: 835–845

Bentsink L, Jowett J, Hanhart CJ, Koornneef M (2006) Cloning of *DOG1*, a quantitative trait locus controlling seed dormancyin *Arabidopsis*. Proc Natl Acad Sci USA **103**: 17042–17047

Borevitz J. Genomic approaches to identifying quantitative trait loci: lessons from *Arabidopsis thaliana*. In: Molecular Genetics and Ecology of Plant Adaptation, pp 53–60, Proceedings of an International Workshop, Vancouver, British Columbia, Canada, **2004** (available at http://naturalvariation.org/)

Borevitz JO, Chory J (2004) Genomics tools for QTL analysis and gene discovery. Curr Opin Plant Biol **7**: 132–136

Borovkova IG, Jin Y, Steffenson BJ (1998) Chromosomal location and genetic relationship of leaf rust resistance genes *Rph9* and *Rph12* in barley. Phytopathology **88**: 76–80

Brueggeman R, Rostoks N, Kudrna D, Kilian A, Han F, Chen J, Druka A, Steffenson B, Kleinhofs A (2002) The barley stem rust-resistance gene *Rpg1* is a novel disease-resistance gene with homology to receptor kinases. Proc Natl Acad Sci USA **99**: 9328–9333

Brugmans B, van der Hulst R, Visser RGF, Lindhout P, van Eck HJ (2003) A new and versatile method for the successful conversion of AFLP markers into simple single locus markers. Nucleic Acids Res **31**: 1–9

Brunner S, Keller B, Feuillet C (2003) A large rearrangement involving genes and low-copy DNA interrupts the microcollinearity between rice and barley at the *Rph7* locus. Genetics **164**: 673–683

Bull J, Mauch F, Hertig C, Rebmann G, Dudler R (1992) Sequence and expression of a wheat gene that encodes a novel protein associated with pathogen defense. Mol Plant-Microbe Interact **5**: 516–519

Caldo RA, Nettleton D, Peng J, Wise RP (2006) Stage-specific suppression of basal defense discriminates barley plants containing fast- and delayed-acting *Mla* powdery mildew resistance alleles. Mol Plant-Microbe Interact **19**: 939–947

Caranta C, Lefebvre V, Palloix A (1997) Polygenic resistance of pepper to potyviruses consists of a combination of isolate-specific and broad-spectrum quantitative trait loci. Mol Plant-Microbe Interact **10**: 872–878.

Cardle L, Ramsay L, Milbourne D, Macaulay M, Marshall D, Waugh R (2000) Computational and experimental characterization of physically clustered simple sequence repeats in plants. Genetics **156**: 847–854

Carollo V, Matthews DE, Lazo GR, Blake TK, Hummel DD, Lui N, Hane DL, Anderson OD (2005) GrainGenes 2.0. An improved resource for the small-grains community. Plant Physiol **139**: 643–651

Catanzariti A-M, Dodds PN, Lawrence GJ, Ayliffe MA, Ellis JG (2006) Haustorially expressed secreted proteins from flax rust are highly enriched for avirulence elicitors. Plant Cell **18**: 243–256

Chen H, Wang S, Xing Y, Xu C, Hayes PM, Zhang Q (2003) Comparative analyses of genomic locations and race specificities of loci for quantitative resistance to *Pyricularia grisea* in rice and barley. Proc Natl Acad Sci USA **100**: 2544–2549.

Chen X, Salamini F, Gebhardt C (2001) A potato molecular-function map for carbohydrate metabolism and transport. Theor Appl Genet **102**: 284–295

Chen Y, Wang H, Wang X, Cao A, Chen P (2006a) Cloning and expression of peroxisomal ascorbate peroxidase gene from wheat. Mol Biol Rep **33**: 207–213

Chen Y, Yang X, He K, Liu M, Li J, Gao Z, Lin Z, Zhang Y, Wang X, Qiu X, Shen Y, Zhang L, Deng X, Luo J, Deng X-W, Chen Z, Gu H, Qu L-J (2006b) The MYB transcription factor superfamily of *Arabidopsis*: expression analysis and phylogenetic comparison with the rice MYB family. Plant Mol Biol **60**: 107–124

Chester KS (1933) The problem of acquired physiological immunity in plants. Q Rev Biol **8**: 275–324

Chicaiza O, Franckowiak JD, Steffenson BJ. New sources of resistance to leaf rust in barley. In: Slinkard AE, Scoles GJ, Rossnagel BG (eds), Proceedings of the 5th Intern Oat Conf & 7th Intern Barley Genet Symp, pp 706–708, Saskatoon, Univ of Saskatchewan, Saskatoon, **1996**

Chisholm ST, Coaker G, Day B, Staskawicz BJ (2006) Host-microbe interactions: shaping the evolution of the plant immune response. Cell **124**: 803–814

Clark JM (1988) Novel non-templated nucleotide addition reactions catalyzed by prokaryotic and eukaryotic DNA polymerases. Nucl Acid Res **16**: 9677–9686

Clarke AE, Knox B (1979) Plants and immunity. Dev Comp Immunol **3**: 571–589

Clifford BC. Barley leaf rust. In: Roelfs AP, Bushnell WR (eds), The Cereal Rusts, Vol. II. Diseases, distribution, epidemiology, and control, pp 173–205, Acad Press, Orlando, **1985**

Coego A, Ramirez V, Ellul P, Mayda E, Vera P (2005) The H_2O_2-regulated *Ep5C* gene encodes a peroxidase required for bacterial speck susceptibility in tomato. Plant J **42**: 283–293

Collins NC, Niks RE, Schulze-Lefert P (2007) Resistance to cereal rusts at the plant cell wall – what can we learn from other host-pathogen systems? Aust J Agr Res: accepted for publication

Cone KC, McMullen MD, Bi IV, Davis GL, Yim Y-S, Gardiner JM, Polacco ML, Sanchez-Villeda H, Fang Z, Schroeder SG, Havermann SA, Bowers JE, Paterson AH, Soderlund CA, Engler FW, Wing RA, Coe jr EH (2002) Genetic, physical, and informatics resources for maize. On the road to an integrated map. Plant Physiol **130**: 1598–1605

Costa JM, Corey A, Hayes PM, Jobet C, Kleinhofs A, Kopisch-Obusch A, Kramer SF, Kudrna D, Li M, Riera-Lizarazu O, Sato K, Szucs P, Toojinda T, Vales MI, Wolfe RI (2001) Molecular mapping of

the Oregon Wolfe Barleys: a phenotypically polymorphic doubled-haploid population. Theor Appl Genet **103**: 415–424

D'Oliveira B (1960) Host range of the aecial stage of *Puccinia hordei* Otth. Melhoramento **13**: 161–88

Da Cunha L, McFall AJ, Mackey D. (2006) Innate immunity in plants: a continuum of layered defenses. Microbes Infect **8**: 1372–1381

Devos KM, Gale MD (2000) Genome relationships: the grass model in current research. Plant Cell **12**: 637–646

Devos G (2005) Updating the 'Crop Circle'. Curr Opin Plant Biol **8**: 155–162

Diab AA (2006) Construction of barley consensus map showing chromosomal regions associated with economically important traits. Afr J Biotechnol **5**: 235–248

Diener AC, Ausubel FM (2005) *RESISTANCE TO FUSARIUM OXYSPORUM 1*, a dominant *Arabidopsis* disease-resistance gene, is not race-specific. Genetics **171**: 305–321

Dilbirligi M, Erayman M, Gill KS (2005) Analysis of recombination and gene distribution in the 2L1.0 region of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.). Genomics **86**: 47–54

Doebley J, Stec A, Hubbard L (1997) The evolution of apical dominance in maize. Nature **386**: 485–488

Doi K, Izawa T, Fuse T, Yamanouchi U, Kubo T, Shimatani Z, Yano M, Yoshimura A (2004) *Ehd1*, a B-type response regulator in rice, confers short-day promotion of flowering and controls *FT-like* gene expression independently of *Hd1*. Genes Dev **18**: 926–936

Dong J, Chen C, Chen Z (2003) Expression profiles of the *Arabidopsis* WRKY gene superfamily during plant defence response. Plant Mol Biol **51**: 21–37

Draper J, Mur LAJ, Jenkins G, Ghosh-Biswas GC, Bablak P, Hasterok R, Routledge APM (2001) *Brachypodium distachyon*. A new model system for functional genomics in grasses. Plant Physiol **127**: 1539–1555

Dros J (1957) The creation and maintenance of two spring barley varieties. Euphytica **6**: 45–48

El-Din El-Assal S, Alonso-Blanco C, Peeters AJM, Raz V, Koornneef M (2001) A QTL for flowering time in *Arabidopsis* reveals a novel allele of *CRY2*. Nat Genet **29**: 435–440

Fan C, Xing Y, Mao H, Lu T, Han B, Xu C, Li X, Zhang Q (2006) *GS3*, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor Appl Genet **112**: 1164–1171

Faris JD, Li WL, Liu DJ, Chen PD, Gill BS (1999) Candidate gene analysis of quantitative disease resistance in wheat. Theor Appl Genet **98**: 219–225

Faville MJ, Vecchies AC, Schreiber M, Drayton MC, Hughes LJ, Jones ES, Guthridge KM, Smith KF, Sawbridge T, Spangenberg GC, Bryan GT, Forster JW (2004) Functionally associated molecular genetic marker map construction in perennial ryegrass (*Lolium perenne* L.). Theor Appl Genet **110**: 2–32

Feuillet C, Schachermayr G, Keller B (1997) Molecular cloning of a new receptor-like kinase gene encoded at the *Lr10* disease resistance locus of wheat. Plant J **11**: 45–52

Franckowiak J D, Jin Y, Steffenson BJ (1997) Recommended allele symbols for leaf rust resistance genes in barley. Barley Genet Newsl **27**: 36–44

Frary A, Nesbitt TC, Frary A, Grandillo S, van der Knaap E, Cong B, Liu J, Meller J, Elber R, Alpert KB, Tanksley SD (2000) *fw2.2*: a quantitative trait locus key to the evolution of tomato fruit size. Science **289**: 85–88

Fridman E, Carrari F, Liu Y-S, Fernie AR, Zamir D (2004) Zooming in on a quantitative trait for tomato yield using interspecific introgressions. Science **305**: 1786–1789

Gao W, Clancy JA, Han F, Jones BL, Budde A, Wesenberg DM, Kleinhofs A, Ullrich SE, North American Barley Genome Project (2004) Fine mapping of a malting-quality QTL complex near the chromosome 4HS telomere in barley. Theor Appl Genet **109**: 750–760

Geffroy V, Sevignac M, De Oliveira JCF, Fouilloux G, Skroch P, Thoquet P, Gepts P, Langin T, Dron M (2000) Inheritance of partial resistance against *Colletotrichum lindemuthianum* in *Phaseolus vulgaris* and co-localization of quantitative trait loci with genes involved in specific resistance. Mol Plant-Microbe Interact **13**: 287–296

Giese H, Holm-Jensen AG, Jensen HP, Jensen J (1993) Localization of the *Laevigatum* powdery mildew resistance to barley chromosome 2 by the use of RFLP markers. Theor Appl Genet **85**: 897–900

Gjetting T, Carver TLW, Skøt L, Lyngkjær MF (2004) Differential gene expression in individual papilla-resistant and powdery mildew-infected barley epidermal cells. Mol Plant-Microbe Interact **17**: 729–738

Gómez-Gómez L, Boller T (2000) *FLS2*: An LRR receptor–like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. Mol Cell **5**: 1003–1011

Gómez-Gómez L (2004) Plant perception systems for pathogen recognition and defence. Mol Immunol **41**: 1055–1062

Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map of barley. Theor Appl Genet **83**: 250–256

Graner A, Streng S, Drescher A, Jin Y, Borovkova I, Steffenson BJ (2000) Molecular mapping of the leaf rust resistance gene *Rph7* in barley. Plant Breeding **119**: 389–392

Gupta PK, Varshney RK (2000) The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica **113**: 163–165

Gutterson N, Reuber TL (2004) Regulation of disease resistance pathways by AP2/ERF transcription factors. Curr Opin Plant Biol **7**: 465–471

Haanstra JPW, Wye C, Verbakel H, Meijer-Dekens F, van den Berg P, Odinot P, van Heusden AW, Tanskley S, Lindhout P, Peleman J (1999) An integrated high-density RFLP-AFLP map of tomato based on two *Lycopersicon esculentum x L. pennellii* F2 populations. Theor Appl Genet **99**: 254–271

Han F, Kleinhofs A, Ullrich SE, Kilian A, Yano M, Sasaki T (1998) Synteny with rice: analysis of barley malting quality QTLs and *rpg4* chromosome regions. Genome **41**: 373–380

Harder DE. Developmental ultrastructure of hyphae and spores. In: Bushnell WR, Roelfs AP (eds), The Cereal rusts, Vol I. Origins, specificities, structure, and physiology, pp 333–373, Academic Press, Orlando, Florida, **1984**

Hasterok R, Marasek A, Donnison IS, Armstead I, Thomas A, King IP, Wolny E, Idziak D, Draper J, Jenkins G (2006) Alignment of the genomes of *Brachypodium distachyon* and temperate cereals and grasses using bacterial artificial chromosome landing with fluorescence *in situ* hybridization. Genetics **173**: 349–362

Heath MC (1974) Light and electron microscope studies of the interactions of host and non-host plants with cowpea rust *Uromyces phaseoli* var. *vignae*. Physiol Plant Pathol **4**: 403–414

Heath MC (1991) Tansley review No 33. Evolution of resistance to fungal parasitism in natural ecosystems. New Phytol **119**: 331–343

Heath MC (1997) Signalling between pathogenic rust fungi and resistant or susceptible host plants. Ann Bot Lond **80**: 713−720

Heath MC (2000) Nonhost resistance and nonspecific plant defenses. Curr Opin Plant Biol **3**: 315−319

Heath MC (2001) Non-host resistance to plant pathogens: non-specific defense or the result of specific recognition events? Physiol Mol Plant P **58**: 53−54

Hiratsuka Y, Sato S. Morphology and taxonomy of rust fungi. In: Scott KJ, Chakravorty (eds), The rust fungi, pp 1−36, Academic Press, New-York, New-York, **1982**

Hoogkamp TJH, Chen W-Q, Niks RE (1998) Specificity of prehaustorial resistance to *Puccinia hordei* and to two inappropriate rust fungi in barley. Phytopathology **88**: 856–861

Hori K, Sato K, Nankaku N, Takeda K (2005) QTL analysis in recombinant chromosome substitution lines derived from a cross between *Hordeum vulgare* ssp. *vulgare* and *Hordeum vulgare* ssp. *spontaneum*. Mol Breeding **16**: 295–311

Hückelhoven R, Kogel K-H (2003) Reactive oxygen intermediates in plant-microbe interactions: who is who in powdery mildew resistance? Planta **216**: 891–902

Ingle RA, Carstens M, Denby KJ (2006) PAMP recognition and the plant-pathogen arms race. BioEssays **28.9**: 880–889

International Rice Genome Sequencing Project (2005) The map-based sequence of the rice genome. Nature **436**: 793–800

Isidore E, van Os H, Andrzejewski S, Bakker J, Barrena I, Bryan GJ, Caromel B, van Eck HJ, Ghareeb B, de Jong W, van Koert P, Lefebvre V, Milbourne D, Ritter E, Rouppe van der Voort JNAM, Rousselle-Bourgeois F, van Vliet J, Waugh R (2003) Toward a marker-dense meiotic map of the potato genome: lessons from linkage group I. Genetics **165**: 2107–2116

Isidore E, Scherrer B, Bellec A, Budin K, Faivre-Rampant P, Waugh R, Keller B, Caboche M, Feuillet C, Chalhoub B (2005) Direct targeting and rapid isolation of BAC clones spanning a defined chromosome region. Funct Integr Genomics **5**: 97–103

Ivandic V, Walther U, Graner A (1998) Molecular mapping of a new gene in wild barley conferring complete resistance to leaf rust (*Puccinia hordei* Otth). Theor Appl Genet **97**: 1235–1239

Ivandic V, Thomas WTB, Nevo E, Zhang Z, Forster BP (2003) Associations of simple sequence repeats with quantitative trait variation including biotic and abiotic stress tolerance in *Hordeum spontaneum*. Plant Breeding **122**: 300–304

Jacobs Th (1989a) The occurrence of cell wall appositions in flag leaves of spring wheats, susceptible and partially resistant to wheat leaf rust. J Phytopathology **127**: 239–249

Jacobs Th (1989b) Haustorium formation and cell wall appositions in susceptible and partially resistant wheat and barley seedlings infected with wheat leaf rust. J Phytopathology **127**: 250–261

Jafary H, Szabo LJ, Niks RE (2006a) Innate nonhost immunity in barley to different heterologous rust fungi is controlled by sets of resistance genes with overlapping specificities. Mol Plant-Microbe Interact **19**: 1270–1279

Jafary H, Albertazi G, Niks RE. Diversity of loci carrying genes for resistance of barley to heterologous rust species. In: Gullner G (eds), Proceedings of the non-specific and specific innate and acquired plant resistance Symp, Plant Protection Institute of the Hungarian Academy of Sciences, Budapest, Hungary, **2006b**

Jander G, Norris SR, Rounsley SD, Bush DF, Levin IM, Last RL (2002) Arabidopsis map-based cloning in the post-genome era. Plant Physiol **129**: 440–450

Jansen C, Korell M, Eckey C, Biedenkopf D, Kogel K-H (2005) Identification and transcriptional analysis of powdery mildew-induced barley genes. Plant Sci **168**: 373–380

Jansen J, de Jong AG, van Ooijen JW (2001) Constructing dense genetic linkage maps. Theor Appl Genet **102**: 1113–1122

Jansen RC (1994) Controlling the type I and type II errors in mapping quantitative trait loci. Genetics **138**: 871–888

Jansen RC, Stam P (1994) High resolution of quantitative traits into multiple loci via interval mapping. Genetics **136**: 1447–1455

Jensen HP, Jørgensen JH (1992) Genetics of '*Laevigatum*' resistance and virulence in barley and powdery mildew. Barley Genet Newsl **21**: 34–37

Jones DA, Takemoto D (2004) Plant innate immunity - direct and indirect recognition of general and specific pathogen-associated molecules. Curr Opin Immunol **16**: 48–62

Kamoun S (2006) A catalogue of the effector secretome of plant pathogenic oomycetes. Annu Rev Phytopathol **44**: 41–60

Karakousis A, Gustafson JP, Chalmers KJ, Barr AR, Langridge P (2003) A consensus map of barley integrating SSR, RFLP, and AFLP markers. Aust J Agric Res **54**: 1173–1185

Keller B, Feuillet C, Yahiaoui N (2005) Map-based isolation of disease resistance genes from bread wheat: cloning in a supersize genome. Genet Res **85**: 93–100

Kicherer S, Backes G, Walther U, Jahoor A (2000) Localising QTLs for leaf rust resistance and agronomic traits in barley (*Hordeum vulgare* L.). Theo Appl Genet **100**: 881–888

Kikuchi S, Satoh K, Nagata T, Kawagashira N, Doi K, Kishimoto N, Yazaki J, Ishikawa M, Yamada H, Ooka H, Hotta I, Kojima K, Namiki T, Ohneda E, Yahagi W, Suzuki K, Li CJ, Ohtsuki K, Shishiki T, Otomo Y, Murakami K, Iida Y, Sugano S, Fujimura T, Suzuki Y, Tsunoda Y, Kurosaki T, Kodama T, Masuda H, Kobayashi M, Xie Q, Lu M, Narikawa R, Sugiyama A, Mizuno K, Yokomizo S, Niikura J, Ikeda R, Ishibiki J, Kawamata M, Yoshimura A, Miura J, Kusumegi T, Oka M, Ryu R, Ueda M, Matsubara K, Kawai J, Carninci P, Adachi J, Aizawa K, Arakawa T, Fukuda S, Hara A, Hashidume W, Hayatsu N, Imotani K, Ishii Y, Itoh M, Kagawa I, Kondo S, Konno H, Miyazaki A, Osato N, Ota Y, Saito R, Sasaki D, Sato K, Shibata K, Shinagawa A, Shiraki T, Yoshino M, Hayashizaki Y (2003) Collection, mapping, and annotation of over 28,000 cDNA clones from *japonica* rice. Science **301**: 376–379

Kleinhofs A, Kilian A, Saghai Maroof MA, Biyashev RM, Hayes P, Chen FQ, Lapitan N, Fenwick A, Blake TK, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells M, Heun M, Franckowiak JD, Hoffman D, Skadsen R, Steffenson BJ (1993) A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. Theor Appl Genet **86**: 705–712

Kleinhofs A, Graner A. An integrated map of the barley genome. In: Philips RL and Vasil IK (eds), DNA-Based Markers in Plants, pp 187–199, Kluwer Academic Publishers, the Netherlands, **2001**

Kojima S, Takahashi Y, Kobayashi Y, Monna L, Sasaki T, Araki T, Yano M (2002) *Hd3a*, a rice ortholog of the *Arabidopsis FT* gene, promotes transition to flowering downstream of *Hd1* under short-day conditions. Plant Cell Physiol **43**: 1096–1105

Kraakman ATW, Martínez F, Mussiraliev B, van Eeuwijk FA, Niks RE (2006) Linkage disequilibrium mapping of morphological, resistance, and other agronomically relevant traits in modern spring barley cultivars. Mol Breeding **17**: 41–58

Kristensen BK, Ammitzbøll H, Rasmussen SK, Nielsen KA (2001) Transient expression of a vacuolar peroxidase increases susceptibility of epidermal barley cells to powdery mildew. Mol Plant Pathol **2**: 311–317

Kroymann J, Donnerhacke S, Schnabelrauch D, Mitchell-Olds T (2003) Evolutionary dynamics of an *Arabidopsis* insect resistance quantitative trait locus. Proc Natl Acad Sci USA **100** (Suppl 2): 14587–14592

Künzel G, Korzun L, Meister A (2000) Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. Genetics **154**: 397–412

Lanaud C, Risterucci AM, Pieretti I, N'Goran JAK, Fargeas D (2004) Characterisation and genetic mapping of resistance and defence gene analogs in cocoa (*Theobroma cacao* L.). Mol Breeding **13**: 211–227

Langridge P, Karakousis A, Collins N, Kretschmer J, Manning S (1995) A consensus linkage map of barley. Mol Breeding **1**: 389–395

Langridge P, Chalmers K. The Principle: Identification and application of molecular markers. In: Lörz H, Wenzel G (eds) Biotechnology in Agriculture and Forestry Vol 55. Molecular Markers Systems, pp 3–22, Springer Verlag, Heidelberg, Germany, **2004**

Leister D, Kurth J, Laurie DA, Yano M, Sasaki T, Devos K, Graner A, Schulze-Lefert P (1998) Rapid reorganization of resistance gene homologues in cereal genomes. Proc Natl Acad Sci USA **95**: 370–375

Leonards-Schippers C, Gieffers W, Schaüfer-Pregl R, Ritter E, Knapp SJ, Salamini F, Gebhardt C (1994) Quantitative resistance to *Phytophthora infestans* in potato: A case study of QTL mapping in an allogamous plant species. Genetics **137**: 67–77

Li JZ, Sjakste TG, Röder MS, Ganal MW (2003) Development and genetic mapping of 127 new microsatellite markers in barley. Theor Appl Genet **107**: 1021–1027

Li Z-K, Luo LJ, Mei HW, Paterson AH, Zhao XH, Zhong DB, Wang YP, Yu XQ, Zhu L, Tabien R, Stansel JW, Ying CS (1999) A "defeated" rice resistance gene acts as a QTL against a virulent strain of *Xanthomonas oryzae* pv. *Oryzae*. Mol Gen Genet **261**: 58–63

Lindhout P (2002) The perspectives of polygenic resistance in breeding for durable disease resistance. Euphytica **124**: 217–226

Liu B, Zhang S, Zhu X, Yang Q, Wu S, Mei M, Mauleon R, Leach J, Mew T, Leung H (2004) Candidate defense genes as predictors of quantitative blast resistance in rice. Mol Plant-Microbe Interact **17**: 1146–1152

Liu J, van Eck J, Cong B, Tanksley SD (2002) A new class of regulatory genes underlying the cause of pear-shaped tomato fruit. Proc Natl Acad Sci USA **99**: 13302–13306

Liu S, Zhang X, Pumphrey MO, Stack RW, Gill BS, Anderson JA (2006) Complex microcolinearity among wheat, rice, and barley revealed by fine mapping of the genomic region harboring a major QTL for resistance to Fusarium head blight in wheat. Funct Integr Genomics **6**: 83–89

Liu ZW, Biyashev RM, Saghai Maroof MA (1996) Development of simple sequence repeat DNA markers and their integration into a barley linkage map. Theor Appl Genet **93**: 869–876

Longo N, Naldini B, Fiordi AC, Tani G, Di Falco P (2006) Host surface tissues and basidiospore-derived infection strategies of some rust fungi. Caryologia **59**: 168–176

Mackey D, McFall AJ (2006) MAMPs and MIMPs: proposed classifications for inducers of innate immunity. Mol Microbiol **61**: 1365–1371

Magnuson VL, Ally DS, Nylund SJ, Karanjawala ZE, Rayman JB, Knapp JI, Lowe AL, Ghosh S, Collins FS (1996) Substrate nucleotide-determined non-templated addition of adenine by *Taq* DNA polymerase: implications for PCR-based genotyping and cloning. BioTechniques **21**: 700–709

Mains EB, Dietz SM (1930) Physiologic forms of barley mildew, *Erysiphe graminis hordei* Marchal. Phytopathology **20**: 229–239

Malysheva-Otto LV, Ganal MW, Röder MS (2006) Analysis of molecular diversity, population structure and linkage disequilibrium in a worldwide survey of cultivated barley germplasm (*Hordeum vulgare* L.). BMC Genet **7**: 6

Mammadov JA, Steffenson BJ, Saghai Maroof MA (2005) High-resolution mapping of the barley leaf rust resistance gene *Rph5* using barley expressed sequence tags (ESTs) and synteny with rice. Theor Appl Genet **111**: 1651–1660

Marcel TC, Niks RE. Molecular dissection of a QTL region for partial resistance to barley leaf rust. In: Spunar J and Janikova J (eds), Proceedings of the 9th Intern Barley Genetics Symp, pp 709–715, Brno, Czech Rep, Agricultural Research Institute Kromeriz, **2004**

Marcel TC, Varshney RK, Barbieri M, Jafary H, de Kock MJD, Graner A, Niks RE (2007) A high-density consensus map of barley to compare the distribution of QTLs for partial resistance to *Puccinia hordei* and of defence gene homologues. Theor Appl Genet **114**: 487–500

Masle J, Gilmore SR, Farquhar GD (2005) The ERECTA gene regulates plant transpiration efficiency in *Arabidopsis*. Nature **436**: 866–870

Matus IA, Hayes PM (2002) Genetic diversity in three groups of barley germplasm assessed by simple sequence repeats. Genome **45**: 1095–1106

Mauch F, Reimmann C, Freydl E, Schaffrath U, Dudler R (1998) Characterization of the rice pathogen-related protein Rir1a and regulation of the corresponding gene. Plant Mol Biol **38**: 577–586

McCouch SR, Teytelman L, Xu Y, Lobos KB, Clare K, Walton M, Fu B, Maghirang R, Li Z, Xing Y, Zhang Q, Kono I, Yano M, Fjellstrom R, DeClerck G, Schneider D, Cartinhour S, Ware D, Stein L (2002) Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res **9**: 199–207

McNeal FH, Konzak CF, Smith EP, Tate WS, Russell TS (1971) A uniform system for recording and processing cereal research data. USDA Agricultural Research Service, Washington, DC, ARS 34-121

Mellersh DG, Heath MC (2001) Plasma membrane-cell wall adhesion is required for expression of plant defense responses during fungal penetration. Plant Cell **13**: 413–424

Mendgen K, Struck C, Voegele RT, Hahn M (2000) Biotrophy and rust haustoria. Physiol Mol Plant P **56**: 141–145

Meyers BC, Kozik A, Griego A, Kuang H, Michelmore RW (2003) Genome-wide analysis of NBS-LRR-encoding genes in *Arabidopsis*. Plant Cell **15**: 809–834

Mohler V, Jahoor A (1996) Allele-specific amplification of polymorphic sites for the detection of powdery mildew resistance loci in cereals. Theor Appl Genet **93**: 1078–1082

Monosi B, Wisser RJ, Pennill L, Hulbert SH (2004) Full-genome analysis of resistance gene homologues in rice. Theor Appl Genet **109**: 1434–1447

Moore G, Devos KM, Wang Z, Gale MD (1995) Grasses, line up and form a circle. Current Biology **5**: 737–739

Moore JH (2005) A global view of epistasis. Nat Genet **37**: 13–14

Morgante M, Hanafey M, Powell W (2002) Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. Nat Genet **30**: 194–200

Morillo SA, Tax FE (2006) Functional analysis of receptor-like kinases in monocots and dicots. Curr Opin Plant Biol **9**: 460–469

Mouchel CF, Briggs GC, Hardtke CS (2004) Natural genetic variation in *Arabidopsis* identifies *BREVIS RADIX*, a novel regulator of cell proliferation and elongation in the root. Genes Dev **18**: 700–714

Muthukrishnan S, Liang GH, Trick HN, Gill BS (2001) Pathogenesis-related proteins and their genes in cereals. Plant Cell Tissue Organ Cult **64**: 93–114

Nakagami H, Pitzschke A, Hirt H (2005) Emerging MAP kinase pathways in plant stress signalling. TRENDS Plant Sci **10**: 339–346

Neervoort WJ, Parlevliet JE (1978) Partial resistance of barley to leaf rust, *Puccinia hordei*. V. Analysis of the components of partial resistance in eight barley cultivars. Euphytica **27**: 33–39

Neff MM, Neff JD, Chory J, Pepper AE (1998) dCAPS, a simple technique for the genetic analysis of single nucleotide polymorphism: Experimental applications in *Arabidopsis thaliana* genetics. Plant J **14**: 387–392

Neu C, Keller B, Feuillet C (2003) Cytological and molecular analysis of the *Hordeum vulgare-Puccinia triticina* nonhost interaction. Mol Plant-Microbe Interact **16**: 626–633

Niks RE (1982) Early abortion of colonies of leaf rust, *Puccinia hordei*, in partially resistant barley seedlings. Can J Bot **60**: 714–723

Niks RE (1983a) Haustorium formation by *Puccinia hordei* in leaves of hypersensitive, partially resistant, and nonhost plant genotypes. Phytopathology **73**: 64–66

Niks RE (1983b) Comparative histology of partial resistance and the nonhost reaction to leaf rust pathogens in barley and wheat seedlings. Phytopathology **73**: 60–63

Niks RE (1986) Failure of haustorial development as a factor in slow growth and development of *Puccinia hordei* in partially resistant barley seedlings. Physiol Mol Plant P **28**: 309–322

Niks RE (1989) Induced accessibility and inaccessibility of barley cells in seedling leaves inoculated with two leaf rust species. J Phytopathology **124**: 296–308

Niks RE, Dekens RG, Van Ommeren A (1989) The abnormal morphology of a very virulent Moroccan isolate belonging or related to *Puccinia hordei*. Plant Dis **73**: 28–31

Niks RE, Fernández E, Van Haperen B, Bekele Aleye B, Martínez F (2000a) Specificity of QTLs for partial and non-host resistance of barley to leaf rust fungi. Acta Phytopathol Hun **35**: 13–21

Niks RE, Waalther U, Jaiser H, Martinez F, Rubiales D, Andersen O, Flath K, Gymer P, Heinrichs F, Jonnsson R, Kuntze L, Rasmussen M, Richter E (2000b) Resistance against barley leaf rust (*Puccinia hordei*) in West-European spring barley germplasm. Agronomie **20**: 769–782

Niks RE, Rubiales D (2002) Potentially durable resistance mechanisms in plants to specialised fungal pathogens. Euphytica **124**: 201–216

Niks RE, Habekuß A, Bekele B, Ordon F (2004) A novel major gene on chromosome 6H for resistance of barley against the barley yellow dwarf virus. Theor Appl Genet **109**: 1536–1543

Nürnberger T, Brunner F, Kemmerling B, Piater L (2004) Innate immunity in plants and animals: striking similarities and obvious differences. Immunol Rev **198**: 249–266

Nürnberger T, Lipka V (2005) Non-host resistance in plants: new insights into an old phenomenon. Mol Plant Pathol **6**: 335–345

O'Connell RJ, Panstruga R (2006) Tête à tête inside a plant cell: establishing compatibility between plants and biotrophic fungi and oomycetes. New Phytol **171**: 699–718

Pajerowska KM, Parker JE, Gebhardt C (2005) Potato homologs of *Arabidopsis thaliana* genes functional in defense signalling-identification, genetic mapping, and molecular cloning. Mol Plant-Microbe Interact **18**: 1107–1119

Panstruga R (2003) Establishing compatibility between plants and obligate biotrophic pathogens. Curr Opin Plant Biol **6**: 320–326

Parida SK, Anand Raj Kumar K, Dalal V, Singh NK, Mohapatra T (2006) Unigene derived microsatellite markers for the cereal genomes. Theor Appl Genet **112**: 808–817

Park RF, Karakousis A (2002) Characterization and mapping of gene *Rph19* conferring resistance to *Puccinia hordei* in the cultivar 'Reka 1' and several Australian barleys. Plant Breeding **121**: 232–236

Parlevliet JE (1975) Partial resistance of barley to leaf rust, *Puccinia hordei*. I. effect of cultivar and development stage on latent period. Euphytica **24**: 21–27

Parlevliet JE (1976) Evaluation of the concept of horizontal resistance in the barley/*Puccinia hordei* host-pathogen relationship. Phytopathology **66**: 494–497

Parlevliet JE, Zadoks JC (1977) The integrated concept of disease resistance: a new view including horizontal and vertical resistance in plants. Euphytica **26**: 5–21

Parlevliet JE (1978) Race-specific aspects of polygenic resistance of barley to leaf rust, *Puccinia hordei*. Neth J Plant Pathol **84**: 121–126

Parlevliet JE, van der Beek JG, Pieters R (1981) Presence in Morocco of brown rust, *Puccinia hordei*, with a wide range of virulence to barley. Cereal Rusts Bull **9**: 3–8

Parlevliet JE, Van Ommeren A (1985) Race specific effects in major genetic and polygenic resistance of barley to barley leaf rust in the field: identification and distribution. Euphytica **34**: 689–695

Parlevliet JE (2002) Durability of resistance against fungal, bacterial and viral pathogens; present situation. Euphytica **124**: 147–156

Paterson AH, DeVerna JW, Lanini B, Tanksley SD (1990) Fine Mapping of quantitative trait loci using selected overlapping recombinant chromosomes, in an interspecies cross of tomato. Genetics **124**: 735–742

Pedley KF, Martin GB (2005) Role of mitogen-active protein kinases in plant immunity. Curr Opin Plant Biol **8**: 541–547

Perovic D, Stein N, Zhang H, Drescher A, Prasad M, Kota R, Kopahnke D, Graner A (2004) An integrated approach for comparative mapping in rice and barley with special reference to the *Rph16* resistance locus. Funct Integr Genomics **4**: 74–83

Pflieger S, Lefebvre V, Causse M (2001) The candidate gene approach in plant genetics: a review. Mol Breeding **7**: 275–291

Phillips RL, Vasil IK (2001) DNA Markers in Plants, Kluwer Academic Publishers, Dordrecht, the Netherlands

Pickering RA, Malyshev S, Künzel G, Johnston PA, Korzun V, Menke M, Schubert I (2000) Locating introgressions of *Hordeum bulbosum* chromatin within the *H. vulgare* genome. Theor Appl Genet **100**: 27–31

Pillen K, Binder A, Kreuzkam B, Ramsay L, Waugh R, Förster J, Leon J (2000) Mapping new EMBL-derived barley microsatellites and their use in differentiating German barley cultivars. Theor Appl Genet **101**: 652–660

Pooni HS, Kearsey MJ (2002) Plant quantitative traits. Encyclopedia of Life Sciences, Nature Publishing Group

Powell W, Machray GC, Provan J (1996) Polymorphism revealed by simple sequence repeats. TRENDS Plant Sci **1**: 215–222

Qi X, Stam P, Lindhout P (1996) Comparison and integration of four barley genetic maps. Genome **39**: 379–394

Qi X, Lindhout P (1997) Development of AFLP markers in barley. Mol Gen Genet **254**: 330–336

Qi X, Stam P, Lindhout P (1998a) Use of locus-specific AFLP markers to construct a high-density molecular map in barley. Theor Appl Genet **96**: 376–384

Qi X, Niks RE, Stam P, Lindhout P (1998b) Identification of QTLs for partial resistance to leaf rust (*Puccinia hordei*) in barley. Theor Appl Genet **96**: 1205–1215

Qi X, Jiang G, Chen W, Niks RE, Stam P, Lindhout P (1999) Isolate-specific QTLs for partial resistance to *Puccinia hordei* in barley. Theor Appl Genet **99**: 877–884

Qi X, Fufa F, Sijtsma D, Niks RE, Lindhout P, Stam P (2000) The evidence for abundance of QTLs for partial resistance to *Puccinia hordei* on the barley genome. Mol Breeding **6**: 1–9

Qi X, Pittaway TS, Lindup S, Liu H, Waterman E, Padi FK, Hash CT, Zhu J, Gale MD, Devos KM (2004) An integrated genetic map and a new set of simple sequence repeat markers for pearl millet, *Pennisetum glaucum*. Theor Appl Genet **109**: 1485–1493

Qu L-J, Zhu Y-X (2006) Transcription factor families in *Arabidopsis*: major progress and outstanding issues for future research. Curr Opin Plant Biol **9**: 544–549

Ramalingam J, Vera Cruz CM, Kukreja K, Chittoor JM, Wu J-L, Lee SW, Baraoidan M, George ML, Cohen MB, Hulbert SH, Leach JE, Leung H (2003) Candidate defense genes from rice, barley, and maize and their association with qualitative and quantitative resistance in rice. Mol Plant-Microbe Interact **16**: 14–24

Ramsay L, Macaulay M, Ivanissevich S degli, MacLean K, Cardle L, Fuller J, Edwards KJ, Tuvesson S, Morgante M, Massari A, Maestri E, Marmiroli N, Sjakste T, Ganal M, Powell W, Waugh R (2000) A Simple Sequence Repeat-based linkage map of barley. Genetics **156**: 1997–2005

Ramsay L, Russell J, Macaulay M, Booth A, Thomas WTB, Waugh R (2004) Variation shown by molecular markers in barley: genomic and genetic constraints. Aspects Appl Biol **72**: 147–154

Remington DL, Ungerer MC, Purugganan MD (2001) Map-based cloning of quantitative trait loci: progress and prospects. Genet Res **78**: 213–218

Ren Z-H, Gao J-P, Li L-G, Cai X-L, Huang W, Chao D-Y, Zhu M-Z, Wang Z-Y, Luan S, Lin H-X (2005) A rice quantitative trait locus for salt tolerance encodes a sodium transporter. Nat Genet **37**: 1141–1146

Reyna NS, Yang Y (2006) Molecular analysis of the rice MAP kinase gene family in relation to *Magnaporthe grisea* infection. Mol Plant-Microbe Interact **19**: 530–540

Rocherieux J, Glory P, Giboulot A, Boury S, Barbeyron G, Thomas G, Manzanares-Dauleux MJ (2004) Isolate-specific and broad-spectrum QTLs are involved in the control of clubroot in *Brassica oleracea*. Theor Appl Genet **108**: 1555–1563

Rohringer R Kim WK, Samborski DJ, Howes NK (1977) Calcofluor: an optical brightener for fluorescence microscopy of fungal plant parasites in leaves. Phytopathology **67**: 808–810

Rossi C, Cuesta-Marcos A, Vales I, Gomez-Pando L, Orjeda G, Wise R, Sato K, Hori K, Capettini F, Vivar H, Chen X, Hayes P (2006) Mapping multiple disease resistance genes using a barley mapping population evaluated in Peru, Mexico, and the USA. Mol Breeding **18**: 355–366

Rostoks N, Mudie S, Cardle L, Russell J, Ramsay L, Booth A, Svensson JT, Wanamaker EM, Hedley PE, Liu H, Morris J, Close TJ, Marshall DF, Waugh R (2005) Genome-wide SNP discovery and linkage analysis in barley based on genes responsive to abiotic stress. Mol Gen Genomics **274**: 515–527

Rouppe van der Voort JNAM, van Zandvoort P, van Eck HJ, Folkertsma RT, Hutten RCB, Draaistra J, Gommers FJ, Jacobsen E, Helder J, Bakker J (1997) Use of allele specificity of comigrating AFLP markers to align genetic maps from different potato genotypes. Mol Gen Genet **255**: 438–447

Rubiales D, Niks RE. Combination of mechanisms of resistance to rust fungi as a strategy to increase durability. In: Royo C, Nachit MM, di Fonzo N, Araus JL (eds), Options méditerranéennes, Durum wheat improvement in the Mediterranean region: New challenges, Proceeding of the seminar jointly organised by CIHEAM, Centre Udl-IRTA, CIMMYT and ICARDA, pp 333–339, Zaragoza, Spain, **2000**

Saghai Maroof MA, Biyashev RM, Yang GP, Zhang Q, Allard RW (1994) Extraordinarily polymorphic microsatellite DNA in barley: Species diversity, chromosomal locations, and population dynamics. Proc Natl Acad Sci USA **91**: 5466–5470

Salvi S, Tuberosa R (2005) To clone or not to clone plant QTLs: present and future challenges. TRENDS Plant Sci **10**: 298–304

Sato K, Nankaku N, Motoi Y, Takeda K. A large scale mapping of ESTs on barley genome. In: Spunar J and Janikova J (eds), Proceedings of the 9th Intern Barley Genetics Symp, pp 79–85, Agricultural Research Institute Kromeriz, Brno, Czech Rep, **2004**

Schaffrath U, Mauch F, Freydl E, Schweizer P, Dudler R (2000) Constitutive expression of the defense-related *Rir1b* gene in transgenic rice plants confers enhanced resistance to rice blast fungus *Magnaporthe grisea*. Plant Mol Biol **43**: 59–66

Schmierer D, Drader T, Horsley R, Kleinhofs A. Saturation mapping of a major fusarium head blight QTL on barley chromosome 2H. In: Canty SM, Lewis J, Ward RW (eds), Proceedings of the National Fusarium Head Blight Forum, pp 36–39, Bloomington, Minnesota, **2003**

See DR, Brooks S, Nelson JC, Brown-Guedira G, Friebe B, Gill BS (2006) Gene evolution at the ends of wheat chromosomes. Proc Natl Acad Sci USA **103**: 4162–4167

Sharopova N, McMullen MD, Schultz L, Schroeder S, Sanchez-Villeda H, Gardiner J, Bergstrom D, Houchins K, Melia-Hancock S, Musket T, Duru N, Polacco M, Edwards K, Ruff T, Register JC, Brouwer C, Thompson R, Velasco R, Chin E, Lee M, Woodman-Clikeman W, Long MJ, Liscum E, Cone K, Davis G, Coe Jr EH (2002) Development and mapping of SSR markers for maize. Plant Mol Biol **48**: 463–481

Shatters Jr RG, Boykin LM, Lapointe SL, Hunter WB, Weathersbee III AA (2006) Phylogenetic and structural relationships of the PR5 gene family reveal an ancient multigene family conserved in plants and select animal taxa. J Mol Evol **63**: 12–29

Shiu S-H, Bleecker AB (2001) Receptor-like kinases from Arabidopsis form a monophyletic gene family related to animal receptor kinases. Proc Natl Acad Sci USA **98**: 10763–10768

Shiu S-H, Bleecker AB (2003) Expansion of the receptor-like kinase/Pelle gene family and receptor-like proteins in *Arabidopsis*. Plant Physiol **132**: 530–543

Shiu S-H, Karlowski WM, Pan R, Tzeng Y-H, Mayer KFX, Lia W-H (2004) Comparative analysis of the receptor-like kinase family in *Arabidopsis* and rice. Plant Cell **16**: 1221–1234

Shtaya MJY, Sillero JC, Rubiales D (2006a) Identification of a new pathotype of *Puccinia hordei* with virulence for the resistance gene *Rph7*. Eur J Plant Pathol **116**: 103–106

Shtaya MJY, Marcel TC, Sillero JC, Niks RE, Rubiales D (2006b) Identification of QTLs for powdery mildew and scald resistance in barley. Euphytica **151**: 421–429

Singh, NK, Dalal V, Batra K, Singh BK, Chitra G, Singh A, Ghazi IA, Yadav M, Pandit A, Dixit R, Singh PK, Singh H, Koundal KR, Gaikwad K, Mohapatra T, Sharma TR (2007) Single-copy genes define a conserved order between rice and wheat for understanding differences caused by duplication, deletion, and transposition of genes. Funct Integr Genomics **7**: 17–35

Sjakste TG, Rashal I, Röder MS (2003) Inheritance of microsatellite alleles in pedigrees of Latvian barley varieties and related European ancestors. Theor Appl Genet **106**: 539–549

Smith H (1978) Recognition and defence in plants. Nature **273**: 266

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet **109**: 1105–1114

Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH (1995) A receptor kinase-like protein encoded by the rice disease resistance gene *Xa21*. Science **270**: 1804–1806

Spaner D, Shugar LP, Choo TM, Falak I, Briggs KG, Legge WG, Falk DE, Ullrich SE, Tinker NA, Steffenson BJ, Mather DE (1998) Mapping of disease resistance loci in barley on the basis of visual assessment of naturally occurring symptoms. Crop Sci **38**: 883–850

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J **3**: 739–744

Stein N, Graner A. Map-based gene isolation in cereal genomes. In: Gupta PK, Varshney RK (eds), Cereal Genomics, pp 331–360, Kluwer Academic Publishers, Dordrecht, the Netherlands, **2004**

Stein N, Prasad M, Scholz U, Thiel T, Zhang H, Wolf M, Kota R, Varshney RK, Perovic D, Grosse I, Graner A (2007) A 1,000-loci transcript map of the barley genome: new anchoring points for integrative grass genomics. Theor Appl Genet: DOI: 10.1007/s00122-006-0480-2

Stephens JL, Brown SE, Lapitan NLV, Knudson DL (2004) Physical mapping of barley genes using an ultrasensitive fluorescence in situ hybridization technique. Genome **47**: 179–189

Steward CN, Via LE (1993) A rapid CTAB DNA isolation technique useful for RAPD fingerprinting and other PCR applications. Biotechniques **14**: 748–750

Struss P, Plieske J (1998) The use of microsatellite markers for detection of genetic diversity in barley populations. Theor Appl Genet **97**: 308–315

Sun X, Cao Y, Yang C, Li X, Wang S, Zhang Q (2004) *Xa26*, a gene conferring resistance to *Xanthomonas oryzae* pv. *oryzae* in rice, encodes an LRR receptor kinase-like protein. Plant J **37**: 517–527

Takahashi Y, Shomura A, Sasaki T, Yano M (2001) *Hd6*, a rice quantitative trait locus involved in photoperiod sensitivity, encodes the a-subunit of protein kinase CK2. Proc Natl Acad Sci USA **98**: 7922–7927

Thiel T, Michalek W, Varshney RK, Graner A (2003) Exploiting EST databases for the development and characterization of gene-derived SSR markers in barley (*Hordeum vulgare* L.). Theor Appl Genet **106**: 411–422

Thomsen SB, Jensen HP, Jensen J, Skou JP, Jørgensen JH (1997) Localization of a resistance gene and identification of sources of resistance to barley leaf stripe. Plant Breeding **116**: 455–459

Thordal-Christensen H (2003) Fresh insights into processes of nonhost resistance. Curr Opin Plant Biol **6**: 351−357

Thornsberry JM, Goodman MM, Doebley J, Kresovich S, Nielsen D, Buckler IV ES (2001) *Dwarf8* polymorphisms associate with variation in flowering time. Nat Genet **28**: 286−289

Thurow C, Schiermeyer A, Krawczyk S, Butterbrodt T, Nickolov K, Gatz C (2005) Tobacco bZIP transcription factor TGA2.2 and related factor TGA2.1 have distinct roles in plant defense responses and plant development. Plant J **44**: 100−113

Tian F, Zhu Z, Zhang B, Tan L, Fu Y, Wang X, Sun CQ (2006) Fine mapping of a quantitative trait locus for grain number per panicle from wild rice (*Oryza rufipogon* Griff.). Theor Appl Genet **113**: 619–629

Torii KU (2004) Leucine-rich repeat receptor kinases in plants: structure, function, and signal transduction pathways. Int Rev Cytol **234**: 1–46

Trognitz F, Manosalva P, Gysin R, Niño-Liu D, Simon R, Rosario Herrera M del, Trognitz B, Ghislain M, Nelson R (2002) Plant defense genes associated with quantitative resistance to potato late blight in *Solanum phureja* x dihaploid *S. tuberosum hybrids*. Mol Plant-Microbe Interact **15**: 587–597

Turner A, Beales J, Faure S, Dunford RP, Laurie DA (2005) The pseudo-response regulator *Ppd-H1* provides adaptation to photoperiod in barley. Science **310**: 1031–1034

Tyler BM, Jiang R, Ferreira A, Wang X, Bruce N, Kale S, Arredondo F, Dou D. W181: Bioinformatics and functional genomics of pathogenicity in the soybean pathogen *Phytophthora sojae*. In: Lazo G, Grant D, Blake V (eds), Final abstracts guide of the Plant and Animal Genome XV conf, pp 45, San Diego, California, **2007**

Van Berloo R, Aalbers H, Werkman A, Niks RE (2001) Resistance QTL confirmed through development of QTL-NILs for barley leaf rust resistance. Mol Breeding **8**: 187–195

Van Berloo R, Hutten RCB (2005) Peditree: pedigree database analysis and visualization for breeding and science. J Hered **96**: 465–468

Van der Plank JE (1963) Plant disease: epidemics and control. Academic Press, New York, London

Van der Plank JE (1968) Disease resistance in plants. Academic Press, NewYork, London

Van Ooijen JW (1992) Accuracy of mapping quantitative trait loci in autogamous species. Theor Appl Genet **84**: 803–811

Van Ooijen JW (1999) LOD significance thresholds for QTL analysis in experimental populations of diploid species. Heredity **83**: 613–624

Van Ooijen JW, Voorrips RE (2001) JoinMap® Version 3.0, Software for the calculation of genetic linkage maps. Plant Research International, Wageningen, the Netherlands

Van Ooijen JW (2004) MapQTL® Version 5, software for the mapping of quantitative trait loci in experimental populations. Kyazma BV, Wageningen, the Netherlands

Van Os H, Stam P, Visser RGF, van Eck HJ (2005a) RECORD: a novel method for ordering loci on a genetic linkage map. Theor Appl Genet **112**: 30–40

Van Os H, Stam P, Visser RGF, van Eck HJ (2005b) SMOOTH: a statistical method for successful removal of genotyping errors from high-density genetic linkage data. Theor Appl Genet **112**: 187–194

Van Os H, Andrzejewski S, Bakker E, Barrena I, Bryan GJ, Caromel B, Ghareeb B, Isidore E, de Jong W, van Koert P, Lefebvre V, Milbourne D, Ritter E, Rouppe van der Voort JNAM, Rousselle-Bourgeois F, van Vliet J, Waugh R, Bakker J, Visser RGF, van Eck HJ (2006) A 10,000 marker ultra-dense genetic recombination map as a new tool for anchoring a physical map and fast gene cloning in potato. Genetics **173**: 1075–1087

Varshney RK, Prasad M, Graner A. Molecular marker maps of barley: a resource for intra- and interspecific genomics. In: Lörz H, Wenzel G (eds), Biotechnology in Agriculture and Forestry Vol 55. Molecular Markers Systems, pp 229–243, Springer Verlag, Heidelberg, Germany, **2004**

Varshney RK, Graner A, Sorrells ME (2005a) Genic microsatellite markers in plants: features and applications. TRENDS Biotech **23**: 48–55

Varshney RK, Sigmund R, Borner A, Korzun V, Stein N, Sorrells ME, Langridge P, Graner A (2005b) Interspecific transferability and comparative mapping of barley EST-SSR markers in wheat, rye and rice. Plant Sci **168**: 195–202

Varshney RK, Grosse I, Hähnel U, Siefken R, Prasad M, Stein N, Langridge P, Altschmied L, Graner A (2006a) Genetic mapping and BAC assignment of EST-derived SSR markers shows non-uniform distribution of genes in the barley genome. Theor Appl Genet **113**: 239–250

Varshney RK, Hoisington DA, Tyagi AK (2006b) Advances in cereal genomics and applications in crop breeding. TRENDS Biotech **24**: 490–499

Varshney RK, Marcel TC, Ramsay L, Russell J, Röder M, Stein N, Waugh R, Langridge P, Niks RE, Graner A (2007) A high density barley microsatellite consensus map with 775 SSR loci. Theor Appl Genet **114**: 1091–1103

Vaz Patto MC, Niks RE (2001) Leaf wax layer may prevent appressorium differentiation but does not influence orientation of the leaf rust fungus *Puccinia hordei* on *Hordeum chilense* leaves. Eur J Plant Pathol **107**: 795–803

Verica JA, Chae L, Tong H, Ingmire P, He Z-H (2003) Tissue-specific and developmentally regulated expression of a cluster of tandemly arrayed cell wall-associated kinase-like kinase genes in *Arabidopsis*. Plant Physiol **133**: 1732–1746

Von Korff M, Wang H, Léon J, Pillen K (2005) AB-QTL analysis in spring barley. I. Detection of resistance genes against powdery mildew, leaf rust and scald introgressed from wild barley. Theor Appl Genet **111**: 583–590

Vos P, Hogers R, Bleeker M, Reijans M, Lee T van de, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res **23**: 4407–4414

Vromans J, Stam P, van Eck HJ (2007) The construction of an integrated linkage map of flax (*Linum usitatissimum* L.) using conventional and novel mapping software. Theor Appl Genet: accepted for publication

Wang H, Qi M, Cutler AJ (1993) A simple method of preparing plant samples for PCR. Nucleic Acid Research **21**: 4153–4154

Wang Z, Taramino G, Yang D, Liu G, Tingey SV, Miao G-H, Wang G-L (2001) Rice ESTs with disease-resistance gene- or defense-response gene-like sequences mapped to regions containing major resistance genes or QTLs. Mol Genet Genomics **265**: 302–310

Wenzl P, Li H, Carling J, Zhou M, Raman H, Paul E, Hearnden P, Maier C, Xia L, Caig V, Ovesná J, Cakir M, Poulsen D, Wang J, Raman R, Smith KP, Muehlbauer GJ, Chalmers KJ, Kleinhofs A, Huttner E, Kilian A (2006) A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. BMC Genom **7**: 206

Werner JD, Borevitz JO, Warthmann N, Trainer GT, Ecker JR, Chory J, Weigel D (2005) Quantitative trait locus mapping and DNA array hybridization identify *FLM* deletion as a cause for natural flowering-time variation. Proc Natl Acad Sci USA **102**: 2460–2465

Xu Y, Zhu L, Xiao J, Huang N, McCouch SR (1997) Chromosomal regions associated with segregation distortion of molecular markers in F_2, backcross, doubled haploid, and recombinant inbred populations in rice (*Oryza sativa* L.). Mol Gen Genet **253**: 535–545

Xu Y, McCouch SR, Zhang Q (2005) How can we use genomics to improve cereals with rice as a reference genome? Plant Mol Biol **59**: 7–26

Yahiaoui N, Srichumpa P, Dudler R, Keller B (2004) Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm3b* from hexaploid wheat. Plant J **37**: 528–538

Yahyaoui AH, Sharp EL, Reinhold M (1988) New sources of resistance to *Puccinia hordei* in barley land race cultivars. Phytopathology **78**: 905–908

Yano M, Katayose Y, Ashikari M, Yamanouchi U, Monna L, Fuse T, Baba T, Yamamoto K, Umehara Y, Nagamura Y, Sasaki T (2000) *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the *Arabidopsis* flowering time gene *CONSTANS*. Plant Cell **12**: 2473–2484

Yu Y, Tomkins JP, Waugh R, Frisch DA, Kudrna D, Kleinhofs A, Brueggeman RS, Muehlbauer GJ, Wise RP, Wing RA (2000) A bacterial artificial chromosome library for barley (*Hordeum vulgare* L.) and the identification of clones containing putative resistance genes. Theor Appl Genet **101**: 1093–1099

Zeyen RJ, Carver TLW, Lyngkjær MF. The formation and role of papillae. In: Bélanger RR, Bushnell WR, Dik AJ, Timothy LW (eds), The Powdery Mildews: A Comprehensive Treatise, pp 107–125, Carver APS Press, St. Paul, Minnesota, **2002**

Zhang S, Chen C, Li L, Meng L, Singh J, Jiang N, Deng X-W, He Z-H, Lemaux PG (2005) Evolutionary expansion, gene structure, and expression of the rice wall-associated kinase gene family. Plant Physiol **139**: 1107–1124

Zhang Y, Wang L (2005) The WRKY transcription factor superfamily: its origin in eukaryotes and expansion in plants. BMC Evol Biol **5**: 1

Zhang Z, Ober JA, Kliebenstein DJ (2006) The gene controlling the quantitative trait locus *EPITHIOSPECIFIER MODIFIER1* alters glucosinolate hydrolysis and insect resistance in *Arabidopsis*. Plant Cell **18**: 1524–1536

Zhong S, Effertz RJ, Jin Y, Franckowiak JD, Steffenson BJ (2003) Molecular mapping of the leaf rust resistance gene *Rph6* in barley and its linkage relationships with *Rph5* and *Rph7*. Phytopathology **93**: 604–609

Zhou M-Y, Clark SE, Gomez-Sanchez CE (1995) Universal cloning method by TA strategy. BioTechniques **19**: 34–35

Zierold U, Scholz U, Schweizer P (2005) Transcriptome analysis of *mlo*-mediated resistance in the epidermis of barley. Mol Plant Pathol **6**: 139–151

Summary

Partial resistance of barley (*Hordeum vulgare*) to the pathogenic barley leaf rust fungus *Puccinia hordei* is based on a non-hypersensitive, pre-haustorial mechanism of defence. There is evidence that the pathogen find it very difficult to adapt itself to partially resistant barley. This is borne out by the observation that the resistance is durably effective, whereas other types of plant resistance can become ineffective by adaptation of the pathogen population. The barley – *P. hordei* relationship serves as a model system for many other plant-pathosystems, where a similar form of resistance exists.

The partial resistance results from the cumulative effect of several genes, each with a small effect. The locations of these genes on the barley chromosomes are called QTLs. The barley cultivar 'Vada' has a high level of partial resistance, which is based on at least six QTLs. Three of them have a relatively large effect. By backcrossing, these three genes have been incorporated into the extremely susceptible line L94 (called Near-isogenic lines, NILs) (**Chapter 4**). We also developed reciprocal NILs incorporating the susceptibility alleles of those QTLs from the L94 into 'Vada' background. These NILs allowed measurement of the effects of the individual genes in a constant genetic background (**Chapters 4 & 5**). We compared the position of QTLs for partial resistance in five barley populations, each consisting of the progeny of two parental barley lines (**Chapter 2**). This indicated a surprisingly large diversity in such genes: only few QTLs that were discovered in one barley accession also contributed to partial resistance in another accession.

Although partial resistance generally is effective to all genotypes of the pathogen species, the NILs proved clearly that individual QTLs may be effective to one but not effective to another strain of the pathogen (**Chapter 5**). Since cultivars with a fair-to-high level of partial resistance carry several QTLs for this trait, ineffectiveness of one of the QTLs to some strain of the pathogen does not lead to complete susceptibility of that cultivar, since the remaining QTLs still restrict the pathogen to a relatively low epidemic development. So, the race-specificity of individual QTLs has no dramatic adverse consequence for the resistance level of the cultivar.

The NILs also allowed to determine the precise position of the QTL with the greatest effect, *Rphq2* (**Chapter 4**). This gene is located at the tip of the long arm of Chromosome 2H where we could pinpoint it to an interval of only about 0.1 cM flanked by two markers. These markers had been developed by using the similarity between barley and rice in that chromosomal region. The availability of the complete DNA sequence of the rice genome helped to find similar sequences in barley at homologous chromosomal regions.

Gene *Rphq2* offers the best perspectives for cloning and sequencing a gene for partial resistance, and hence, understanding its molecular function in plant defence to such a

specialised pathogen as *P. hordei*. The gene has a relatively large phenotypic effect on the resistance level of barley to *P. hordei* and is located in a chromosomal region with a high frequency of recombination. Each centiMorgan on the linkage map around the gene corresponds probably with only a relatively short stretch of DNA. This makes it feasible to pick the gene up from a bacterial DNA (BAC) library in not too distant future.

The fine-mapping of *Rphq2* involved several molecular and genetic strategies. During that work it turned out to be feasible and useful to integrate linkage maps of six barley mapping populations (**Chapters 2 & 3**). Each of those populations had been constructed on the basis of RFLP markers, AFLP markers and/or SSR markers. Quite many markers segregated in more than one of the six populations, allowing combination of those maps into one new, integrated map, consisting of over 3,200 markers (**Chapter 2**). This is the highest density marker map of barley published thus far. Important in this integration work was to determine the map position of SSR markers in (additional) mapping populations and the development of new SSR markers. This class of markers is highly polymorphic and technically easy to apply. Combining SSR marker data from several laboratories, including ours, resulted in the construction of a consensus map, where map positions of almost 800 SSR marker loci have been put together (**Chapter 3**). The incorporation of many markers based on gene or EST sequences in the constructed consensus maps provided a means to compare the distribtution of QTLs with the distribution of defence gene homologues. It led us to the identification of candidate genes to explain the genetic basis of partial resistance.

Samenvatting

Partiële resistentie van gerst (*Hordeum vulgare*) tegen de pathogene dwergroestschimmel *Puccinia hordei* berust op een niet-overgevoeligheidsreactie vóór de vorming van haustoria in de plantcel. Kennelijk is het voor het pathogeen moeilijk zich aan te passen aan partieel resistente gerst. Dat blijkt uit het feit dat de resistentie duurzaam effectief is, terwijl andere soorten plantresistentie ineffectief kunnen worden doordat de schimmel populatie zich wel aanpast. De gerst – *P. hordei* relatie dient als modelsysteem voor vele andere plant-pathogeencombinaties, waar een dergelijke resistentievorm ook voorkomt.

De partiële resistentie wordt veroorzaakt door het cumulatieve effect van verscheidene genen, die elk een klein effect hebben. De plaatsen waar deze genen zich op de gerstchromosomen bevinden worden QTLs genoemd. De gerstcultivar 'Vada' heeft een hoog niveau van partiële resistentie, welke gebaseerd is op een combinatie van tenminste zes QTLs. Drie ervan hebben een relatief groot effect op het niveau van partiële resistentie. Door middel van terugkruising werden deze drie genen ingebracht in de zeer vatbare gerstlijn L94, wat resulteerde in bijna-isogene lijnen (NILs) (**Hoofdstuk 4**). We ontwikkelden ook de reciproke NILs, waarin de vatbaarheidsallelen van die QTLs uit L94 werden ingebracht in de genetische achtergrond van 'Vada'. Deze NILs werden gebruikt om het effect van de individuele genen te bepalen in een constante genetische achtergrond (**Hoofdstukken 4 & 5**). We vergeleken de positie van QTLs voor partiële resistentie in vijf populaties van gerst, die elk de nakomelingschappen waren van twee ouders (**Hoofdstuk 2**). Hieruit bleek dat er een verrassende diversiteit in genen bestaat die bijdragen aan partiële resistentie: slechts enkele van de QTLs die ontdekt werden in de ene gerstlijn droegen ook in een andere gerstlijn bij aan partiële resistentie.

Hoewel partiële resistentie in het algemeen effectief is tegen alle genotypen van de pathogene schimmel, toonden de resultaten met de NILs duidelijk aan dat individuele QTLs effectief tegen het ene genotype van de schimmel en ineffectief tegen andere genotypen kunnen zijn (**Hoofdstuk 5**). Omdat gerstrassen met een behoorlijk niveau van partiële resistentie meestal verscheidene QTLs voor die eigenschap bezet hebben met het resistentieallel, impliceert ineffectiviteit van één van de QTLs tegen een of andere variant van de schimmel niet dat de plant dan volledig vatbaar wordt. De overige QTLs beperken nog steeds het pathogeen en de epidemische uitbreiding daarvan. Daardoor is de specificiteit van individuele QTLs voor varianten van de schimmel niet direct dramatisch voor het resistentieniveau van dat gerstras.

De NILs hielpen ook om de precieze positie te bepalen van het QTL met het grootste effect, *Rphq2* (**Hoofdstuk 4**). Dit gen is gesitueerd bij de top van de lange arm van Chromosoom 2H, waar we het konden lokaliseren in een stukje van ongeveer 0.1 cM,

geflankeerd door twee merkers. Deze merkers hebben we ontwikkeld door gebruik te maken van de gelijkenis in DNA tussen gerst en rijst voor dat chromosoomsegment. De beschikbaarheid van de volledige DNA basenvolgorde van rijst was nuttig om soortgelijke basenvolgordes te vinden in het gerst DNA op homologe chromosoomsegmenten.

Gen *Rphq2* biedt de beste perspectieven voor isolatie en basenvolgordebepaling van een gen voor partiële resistentie, en daardoor voor begrip van de moleculaire functie daarvan in de verdediging van de plant tegen gespecialiseerde micro-organismen als *P. hordei*. Het gen heeft een relatief hoog fenotypisch effect op het niveau van resistentie van gerst tegen *P. hordei* en is gesitueerd in een chromosoomsegment met een hoge frequentie van recombinatie. Dat betekent dat elke centiMorgan op de genetische kaart rond het gen waarschijnlijk correspondeert met slechts een relatief kort stukje DNA-sequentie. Dit moet het in de nabije toekomst mogelijk maken het gen te vinden in een bacteriële DNA (BAC) bibliotheek.

Voor de fijn-kartering van *Rphq2* werden verscheidene moleculaire en genetische strategieën toegepast. Daarbij bleek dat het mogelijk en gewenst was om de genetische koppelingskaarten van zes gerstpopulaties te integreren (**Hoofdstukken 2 & 3**). Elk van die kaarten waren gebaseerd op RFLP, AFLP en/of SSR merkers. Vrij veel van de merkers splitsten uit in meer dan een van de zes populaties, wat het mogelijk maakte de kaarten te combineren tot een nieuwe, geïntegreerde kaart, die meer dan 3200 merkerloci omvat (**Hoofdstuk 2**). Dit is de meest merker-dichte genetische kaart van gerst die op dit moment gepubliceerd is. Het was voor dit werk belangrijk om de kaartpositie van SSR merkers te bepalen in additionele populaties, en om nieuwe SSR merkers te ontwikkelen. Dit type merker is namelijk zeer polymorph en technisch eenvoudig toe te passen. De combinatie van SSR merkergegevens van verscheidene laboratoria, inclusief het onze, resulteerde in de ontwikkeling van een consensus genetische kaart waarop de posities van bijna 800 SSR merkerloci zijn samengebracht (**Hoofdstuk 3**). De uitbreiding van de merkerdatasets met merkers die gebaseerd zijn op gen- of ESTsequenties verschaft mogelijkheden de distributie van QTLs over het genoom te vergelijken met de distributie van “defence gene homologues”. Dit zijn sequenties met homologie met genen die betrokken zijn bij resistentiereacties van planten tegen pathogenen. Deze vergelijking leidde tot de identificatie van genen die wellicht een rol spelen in de genetische basis van partiële resistentie.

Résumé

La résistance partielle de l'orge (*Hordeum vulgare*) au champignon pathogène de la rouille des feuilles de l'orge *Puccinia hordei* est basée sur un mécanisme de défense pré-haustorial qui n'est pas lié à une réaction d'hypersensibilité. Il a été constaté que le pathogène a beaucoup de difficultés à s'adapter à des lignées d'orge partiellement résistantes. Cela se traduit par le fait que la résistance partielle a un effet durable alors que d'autres types de résistance des plantes aux maladies deviennent inefficaces après adaptation du pathogène. La relation orge – *P. hordei* sert de système modèle pour plusieurs autres plante-pathosystèmes dans lesquels des formes de résistance similaires existent.

La résistance partielle est le résultat de l'effet cumulatif de plusieurs gènes, chacun d'entre eux ayant un effet réduit. Les positions de ces gènes sur les chromosomes de l'orge sont appelées QTLs. Le cultivar d'orge « Vada » a un niveau de résistance partielle élevé qui découle de l'effet d'au moins six QTLs. Trois d'entre eux ont un effet relativement large. Par rétrocroisement, ces trois gènes ont été incorporés dans la lignée sensible L94 résultant en des lignées presque isogéniques (NILs) (**Chapitre 4**). Nous avons aussi développé les NILs réciproques en incorporant les allèles sensibles de ces QTLs, provenant de L94, dans le fond génétique de « Vada ». Ces NILs ont permis de mesurer les effets de chaque gène individuellement, dans un fond génétique constant (**Chapitres 4 & 5**). Nous avons comparé la position des QTLs de résistance partielle provenant de cinq populations d'orge, chaque population étant constituée de la descendance de deux lignées parentales d'orge (**Chapitre 2**). Cette comparaison a révélé un nombre et une diversité surprenante de gènes : seulement quelques QTLs, qui ont été découverts dans une accession d'orge, contribuaient également au niveau de résistance partielle d'une autre accession.

Même si la résistance partielle est globalement efficace contre tous les génotypes d'une même espèce de pathogène, les NILs ont clairement démontré que, individuellement, les QTLs peuvent avoir un effet contre une souche du pathogène et être inefficaces contre une autre souche de ce même pathogène (**Chapitre 5**). Puisque les cultivars avec un niveau de résistance partielle élevé possèdent généralement plusieurs QTLs pour ce caractère, l'absence d'effet d'un QTL contre une ou plusieurs souches du pathogène ne conduit pas à une sensibilité complète de ce cultivar ; les QTLs restants continuent de restreindre le développement épidémique du pathogène. Par conséquent, l'effet spécifique des QTLs contre certaines souches d'un même pathogène n'a pas de conséquence dramatique sur le niveau global de résistance du cultivar.

Les NILs ont également permis de déterminer la position précise du QTL ayant l'effet le plus important, *Rphq2* (**Chapitre 4**). Ce gène est situé à l'extrémité du bras long du Chromosome 2H sur lequel nous avons pu le localiser dans un intervalle d'à peu près 0.1 cM,

flanqué par deux marqueurs moléculaires. Ces marqueurs ont été développés en utilisant la ressemblance entre l'orge et le riz dans cette région de chromosome. La disponibilité de la séquence ADN complète du génome du riz a aidé à l'identification de séquences d'orge similaires dans la région de chromosome homologue.

Le gène *Rphq2* offre la meilleur chance de cloner et de séquencer un gène de résistance partielle, et ainsi, de comprendre sa fonction moléculaire dans la défense des plantes contre les pathogènes spécialistes tels que *P. hordei*. Ce gène a un effet phénotypique relativement important sur le niveau de résistance de l'orge à *P. hordei* et est situé dans une région de chromosome où la fréquence de recombinaison est élevée. A proximité du gène, chaque centiMorgan de la carte génétique correspond probablement à un fragment d'ADN relativement court. Cela rend réalisable l'identification du gène à partir d'une banque de grands fragments d'ADN (banque BAC) dans un futur proche.

La cartographie fine de *Rphq2* a impliqué plusieurs stratégies moléculaires et génétiques. Durant ces travaux, il s'est avéré possible et utile d'intégrer les cartes génétiques de six populations d'orge (**Chapitres 2 & 3**). Les cartes génétiques de chacune de ces populations ont été construites avec des marqueurs RFLP, AFLP et/ou SSR. Un nombre conséquent de ces marqueurs était en ségrégation dans plusieurs populations, ce qui a permis de combiner leurs cartes génétiques en une nouvelle carte, intégrée, contenant plus de 3.200 marqueurs (**Chapitre 2**). Parmi les cartes génétiques d'orge publiées à ce jour, cette nouvelle carte intégrée est celle qui a la plus importante densité de marqueurs. Pendant ce travail d'intégration, il s'est avéré primordial de déterminer la position génétique de marqueurs SSR dans plusieurs populations et de développer de nouveaux marqueurs SSR. Cette catégorie de marqueur est très polymorphe et techniquement facile à utiliser. La combinaison des données de marqueurs SSR provenant de plusieurs laboratoires, y compris le nôtre, a résulté dans la construction d'une autre carte intégrée dans laquelle est donnée la position de près de 800 lieus de marqueurs SSR (**Chapitre 3**). L'incorporation dans ces cartes génétiques intégrées de marqueurs développés à partir de séquences de gène ou d'EST a permis de comparer la dispersion des QTLs avec la dispersion d'homologues de gènes de défense. Cela a rendu possible l'identification de gènes candidats pour expliquer la base génétique de la résistance partielle.

Acknowledgements

I am glad to acknowledge everyone who has contributed to the build-up of my scientific personality and to the realisation of this thesis. Without your help and advices it would have been impossible to produce this work.

I had my first experience in a research institution during a training period that I realised at Fairchild Tropical Garden, in Miami. I really appreciated the valuable time that **Dena Garvue** and **Dr. Scott Zona** spent with me. It surely has something to do with my affinity with sciences today.

Few years later, I realised my Master thesis in Wageningen. I learned so much with **Dr. Yuling Bai**, **Ir. Ron van der Hulst** and **Dr. Pim Lindhout**. With you, I made my first steps in a laboratory and in the genetics of plant resistance to diseases. Then, **Dr. Rients E. Niks** proposed me to stay in Wageningen to perform a PhD thesis. That was such a tough decision for me to make but I decided to take the challenge. **Rients**, I will never forget how happy you was the day I decided to work with you. Since then you have never stopped believing in me and encouraging me. This is little to say that I could never have realised this work and write this thesis without your support, your ideas and your suggestions. I wish to always keep the same scientific enthusiasm that you have. I can't thank you enough for all that you have done.

I really enjoyed to work and interact with many students during my work. All of you were so different from each other and came to Wageningen with various expectations. You all contributed to this thesis by your work and your ideas. **Fabien Marcel**, you came here for one month only but it was such a pleasure to share your first steps in plant genetics. I wish you to become a great scientist. **Benoit Gorguet**, **Minh Truong Ta**, **Mirko Barbieri**, **Jérôme Durand**, each of you is co-author of a chapter of this thesis and I am proud to see that you all decided to follow a scientific path by starting your own PhD research. **Mumta Chhetri**, **Simon Duquesne**, **Vincent Hennette**, you took different directions but you all acknowledged what you have learned with us. **Gonzalo Remiro Rodenas**, I am glad that you found a position at the Spanish Foundation for Science and Technology that suits you so well. **Thijs van Dijk**, **Alice Lorriaux**, I so much appreciated your efficiency and the energy you put into your work. Thank you all for your inputs.

I also had the pleasure during my PhD to share my supervisor with **Dr. Hossein Jafary** and **Reza Aghnoum**. We have formed a very nice "*barley team*" together and I am glad that we could so nicely interact and help each other during our work. I also learned a lot about Iran with you but, above all, I will always remember your integrity, your attentiveness

and your kindness. **Dr. Fernando Martínez**, you have been part of our "*barley team*" as well and it is always a pleasure to meet you again. **Dr. Ana Maria Gonzalez** and **Zuzana Kohutova** you are now part of our "*barley team*" and bring to it so much positive energy. **Anton Vels**, your help in the greenhouse and with the field experiments has been invaluable. **Benoit Gorguet**, we are colleagues and have done our PhD in the same time. I am often impressed by your scientific quality. But what I value the most is my friendship to you and **Adriana**. Our plant resistance working group has been a very friendly group to work with, with many interesting scientific discussions and challenging questions. I would like to thank my promoter, **Prof. Dr. Richard G.F. Visser**, to make our work possible. Our Laboratory could never run smoothly without the carefull attention of **Fien Meijer-Dekens**, **Petra van den Berg**, **Koen Pelgrom** and all other technicians. As well, our Department could not run without the work of **Annie Marchal** and **Letty Dijker**. Thank you all for your help.

I also would like to take this opportunity to tell my gratitude to **Dr. Rajeev K. Varshney** from ICRISAT (India) who has contributed so much to the present thesis and to **Dr. Boulos Chalhoub** from URGV (France) for his recent contribution to the progress of our research.

I am a really lucky person to have such a caring family. My parents always supported me in any decision I took. **My father** helped me more than he might think to find my way in life and **my mother** has always been so proud for anything I could realise. I remember the day when I first suggested to start PhD studies and both of you just told me to follow what I like and that, anyway, you would always be there for me. The only way for me to thank you enough is to be as attentive with my own son as you were with me. **My brother** is so special to me; I really value our relationship. I will never forget **my grand-mother** whose smile is for ever in my heart. Finally, the Netherlands have brought me the most wonderful thing in my life: my wife, **Hoa**, and my son, **Brian**. I love you so much more than I could ever say.

Curriculum vitae

Thierry C. Marcel was born in the year 1977 in Saint-Germain-en-Laye, Yvelines, France. After completing his academic high school in 1996, he joined ISTOM, close to Paris, where he studied *agronomic engineering* and *international agro-development*. He graduated in 2000 and obtained the DESTOM diploma. During his studies at ISTOM, he performed a 3-months program at Larenstein school in Deventer (the Netherlands), where he obtained a degree of specialisation in *tropical crop production* (2000). At ISTOM, he also had the opportunity to do several training periods: at Fairchild Tropical Garden, in Miami (USA), he participated in programs of *conservation of endangered plant species* (1998); and at Tropicasem, a breeding company for tropical areas, in Dakar (Senegal), he studied the *organisation and limitations of market gardening chains in Senegal* (1999).

Subsequently, Thierry followed an MSc degree in plant sciences with specialisation in plant breeding at Wageningen University (the Netherlands). He obtained his MSc degree with distinction in January 2002. The MSc thesis was realised at the Department of Plant Breeding from Wageningen University under the supervision of Dr. Bai Yuling, Ir. Ron van der Hulst and Dr. Pim Lindhout. The work realised during this thesis concerned the *genetic mapping of genes for resistance to powdery mildew in tomato*. This thesis was rewarded by the C.T. de Wit academic price in December 2002. Then, in April 2002, he started his PhD research at the Departement of Plant Breeding from Wageningen University, sponsored by the Dutch Organisation for Scientific Research – Earth and Life Sciences. During his PhD, he studied the *genetics of the interaction between barley (Hordeum vulgare) and leaf rust (Puccinia hordei)* under the supervision of Dr. Rients E. Niks. This thesis presents the outcome of his PhD research. Since May 2006, he obtained a PostDoc position to carry on his research on *partial resistance in the barley – leaf rust plant-pathosystem*, supported by the European Commission through the 6th framework programme's integrated project, BioExploit.

List of publications

Bai Y, van der Hulst R, Huang CC, Wie L, Stam P, Lindhout P (2004) Mapping *Ol-4*, a gene conferring resistance to *Oidium neolycopersici* and originating from *Lycopersicon peruvianum* LA2172, requires multi-allelic, single-locus markers. Theor Appl Genet **109**: 1215–1223. Marcel TC was acknowledged for assistance

Bai Y, van der Hulst R, Bonnema G, Marcel TC, Meijer-Dekens F, Niks RE, Lindhout P (2005) Tomato defense to *Oidium neolycopersici*: dominant *Ol* genes confer isolate-dependent resistance via a different mechanism than recessive *ol-2*. Mol Plant – Microbe Interact **18**: 354–362

Kraakman ATW, Martínez F, Mussiraliev B, van Eeuwijk FA, Niks RE (2006) Linkage disequilibrium mapping of morphological, resistance, and other agronomically relevant traits in modern spring barley cultivars. Mol Breeding **17**: 41–58. Marcel TC was acknowledged for SSR analyses

Shtaya MJY, Marcel TC, Sillero JC, Niks RE, Rubiales D (2006) Identification of QTLs for powdery mildew and scald resistance in barley. Euphytica **151**: 421–429

Marcel TC, Niks RE. Molecular dissection of a QTL region for partial resistance to barley leaf rust. In: Spunar J and Janikova J (eds), Proceedings of the 9th Intern Barley Genetics Symp, pp 709–715, Brno, Czech Rep, Agricultural Research Institute Kromeriz, **2004**

Jafary H, Szabo LJ, Niks RE (2006a) Innate nonhost immunity in barley to different heterologous rust fungi is controlled by sets of resistance genes with overlapping specificities. Mol Plant-Microbe Interact **19**: 1270–1279. Marcel TC was acknowledged for his help in construction of the genetic map

Marcel TC, Varshney RK, Barbieri M, Jafary H, de Kock MJD, Graner A, Niks RE (2007) A high-density consensus map of barley to compare the distribution of QTLs for partial resistance to *Puccinia hordei* and of defence gene homologues. Theor Appl Genet **114**: 487–500

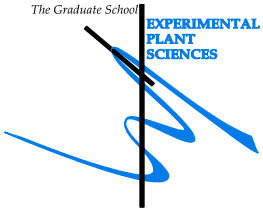
Jiang G-L, Marcel TC, Martínez F, Niks RE (2007) Relative ratio of mature pustules: a simple method to assess partial resistance of barley to *Puccinia hordei*. Plant Dis **91**:301–307

Varshney RK, Marcel TC, Ramsay L, Russell J, Röder M, Stein N, Waugh R, Langridge P, Niks RE, Graner A (2007) A high density barley microsatellite consensus map with 775 SSR loci. Theor Appl Genet **114**:1091–1103

Marcel TC, Aghnoum R, Durand J, Varshney RK, Niks RE. Dissection of the barley 2L1.0 region carrying the '*Laevigatum*' quantitative resistance gene to leaf rust using near isogenic lines (NILs) and sub-NILs. Submitted

Jafary H, Albertazzi G, Marcel TC, Niks RE. High diversity of genes for nonhost immunity of barley to heterologous rust fungi. Submitted

Education Statement of the Graduate School
Experimental Plant Sciences



Issued to: **Thierry C. Marcel**
Date: **11 May 2007**
Group: **Laboratory of Plant Breeding, Wageningen University**

1) Start-up phase	*date*
► **First presentation of your project**	
Effects of individual QTLs on partial resistance of barley to various leaf rust isolates	11 Oct 2002
► **Writing or rewriting a project proposal**	
► **Writing a review or book chapter**	
► **MSc courses**	
PBR-50303: Linkage analysis and mapping software (Prof. P. Stam, WUR)	13-17 May 2002
► **Laboratory use of isotopes**	
Safety with Radioactivity (Luc Suurs): exam passed	18-20 Mar 2003
Subtotal Start-up Phase	*6.0 credits**

2) Scientific Exposure	*date*
► **EPS PhD student days**	
EPS PhD student day 2003 (Utrecht): attendance	27 Mar 2003
EPS PhD student day 2005 (Nijmegen): attendance	02 Jun 2005
► **EPS theme symposia**	
EPS Theme 2 Symposia 2003: attendance, presentation	12 Dec 2003
► **NWO Lunteren days and other National Platforms**	
NWO-ALW meeting Lunteren 2002: attendance	01-02 Apr 2002
NWO-ALW meeting Lunteren 2003: attendance, poster	07-08 Apr 2003
NWO-ALW meeting Lunteren 2004	05-06 Apr 2004
NWO-ALW meeting Lunteren 2005	04-05 Apr 2005
NWO-ALW meeting Lunteren 2006: attendance, poster	03-04 Apr 2006
► **Seminars (series), workshops and symposia**	
Seminar Dr. Jens Kossmann: Plant research at Risoe National Laboratory	21 Mar 2003
Seminar Dr. Levente Kiss: New approaches to understand the evolution of powdery mildew fungi	28 Sep 2004
Seminar Dr. Rajeev Varshney: Functional molecular markers in barley: development and applications	02 Dec 2004
Seminar Prof. Nicola Pecchioni: QTLs and candidate genes for acclimatisation-related traits in the Nure x Tremois barley cross	07 Dec 2004
Seminar Prof. Dr. Sophien Kamoun: Reprogramming the host: The effector secretome of Phytophthora infestans	05 Oct 2005
Seminar Prof.dr. Masahiro Yano: Uncovering genetic control of flowering time in rice	26 Jun 2006
► **Seminar plus**	
► **International symposia and congresses**	
Agrogene Seminar (Paris, France)	27-28 Feb 2003
9th International Barley Genetics Symposium (Brno, Czech Republic)	20-26 Jun 2004
11th International Cereal Rusts & Powdery Mildew Conference (Norwich, England)	22-27 Aug 2004
Plant & Animal Genome XIV Conference (San Diego, United-States)	14-18 Jan 2006
Plant & Animal Genome XV Conference (San Diego, United-States)	13-17 Jan 2007
► **Presentations**	
Disease Resistance in Plants (Wageningen): presentation	14-16 Oct 2002
EPS Theme 2 Symposia 2003: presentation	12 Dec 2003
9th International Barley Genetics Symposium (Brno, Czech Republic): poster & paper	20-26 Jun 2004
11th International Cereal Rusts & Powdery Mildew Conference (Norwich, England): presentation	22-27 Aug 2004
Plant & Animal Genome XIV Conference (San Diego, United-States): poster	14-18 Jan 2006
NWO-ALW meeting Lunteren 2006: poster	03-04 Apr 2006
Plant & Animal Genome XV Conference (San Diego, United-States): presentation	13-17 Jan 2007
► **IAB interview**	01 Jun 2005
► **Excursions**	
Breeding Company Nunhems	May 2006
Subtotal Scientific Exposure	*16.3 credits**

3) In-Depth Studies	*date*
► **EPS courses or other PhD courses**	
Disease Resistance in Plants (Wageningen)	14-16 Oct 2002
Functional Genomics (Utrecht)	25-28 Aug 2003
Signaling in Plant Development and Defence: towards Systems Biology (Wageningen)	19-21 Jun 2006
Gateway to Gateway Technology (Wageningen)	20-24 Nov 2006
► **Journal club**	
member of a literature discussion group at the Plant Breeding Group	2002-2006
► **Individual research training**	
Subtotal In-Depth Studies	*7.2 credits**

4) Personal development	*date*
► **Skill training courses**	
PhD Scientific Writing (CENTA, Wageningen)	21 May-05 Jul 2005
► **Organisation of PhD students day, course or conference**	
Organisation of the Lab-Trip of the Laboratory of Plant Breeding and PRI groups	01 Jun 2006
► **Membership of Board, Committee or PhD council**	
Subtotal Personal Development	*3.3 credits**

TOTAL NUMBER OF CREDIT POINTS*	**32.8**

Herewith the Graduate School declares that the PhD candidate has complied with the educational requirements set by the Educational Committee of EPS which comprises of a minimum total of 30 credits

The work described in this thesis was performed with the Graduate School of Experimental Plant Sciences at the Laboratory of Plant Breeding of Wageningen University. This research was financially supported by the Dutch Organisation for Scientific Research (NWO Aard- en Levenswetenschappen, project no. 809.36.001) through the project “Genetic dissection of prehaustorial resistance to leaf rust (*Puccinia hordei*) in barley (*Hordeum vulgare*)”.

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Front cover:

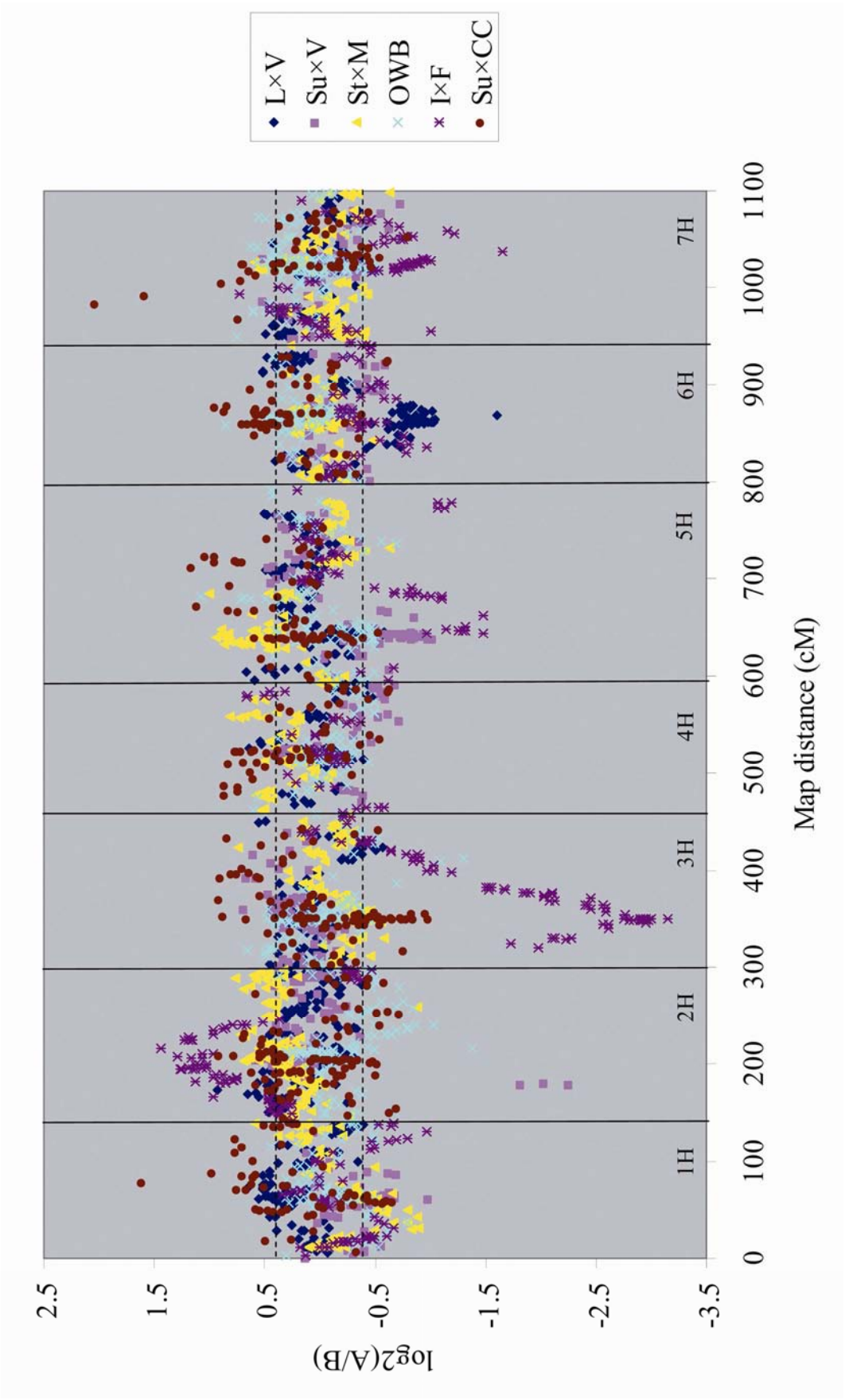
Barley cultivar ‘Steptoe’ infected with *Puccinia hordei* Otth.

Back cover:

Hordeum vulgare L. (common barley).
USDA-NRCS PLANTS Database / Hitchcock, A.S. (rev. A. Chase). 1950**.** *Manual of the grasses of the United States***.** USDA Misc. Publ. No. 200. Washington, DC.

COLOUR FIGURES

Front cover



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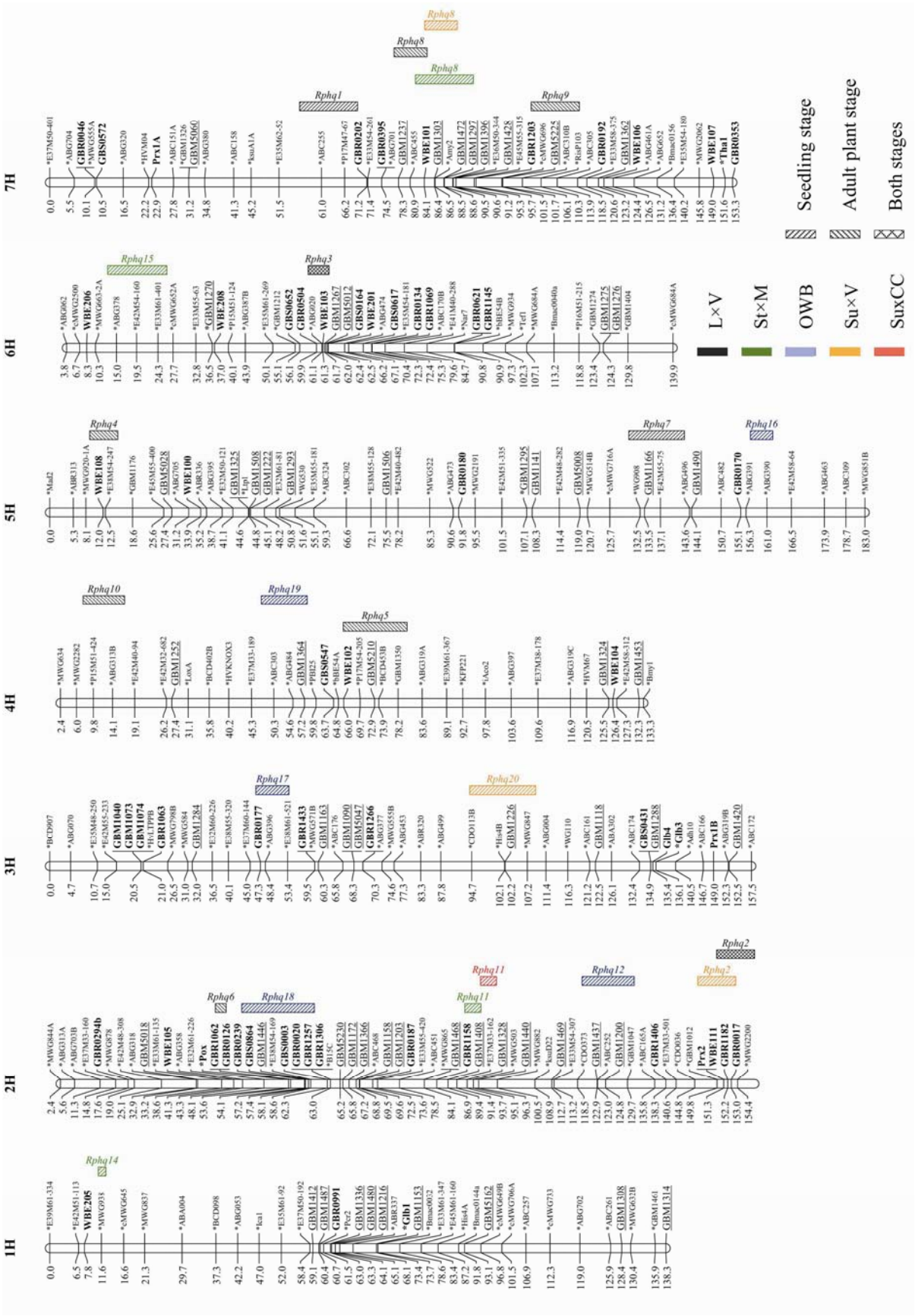

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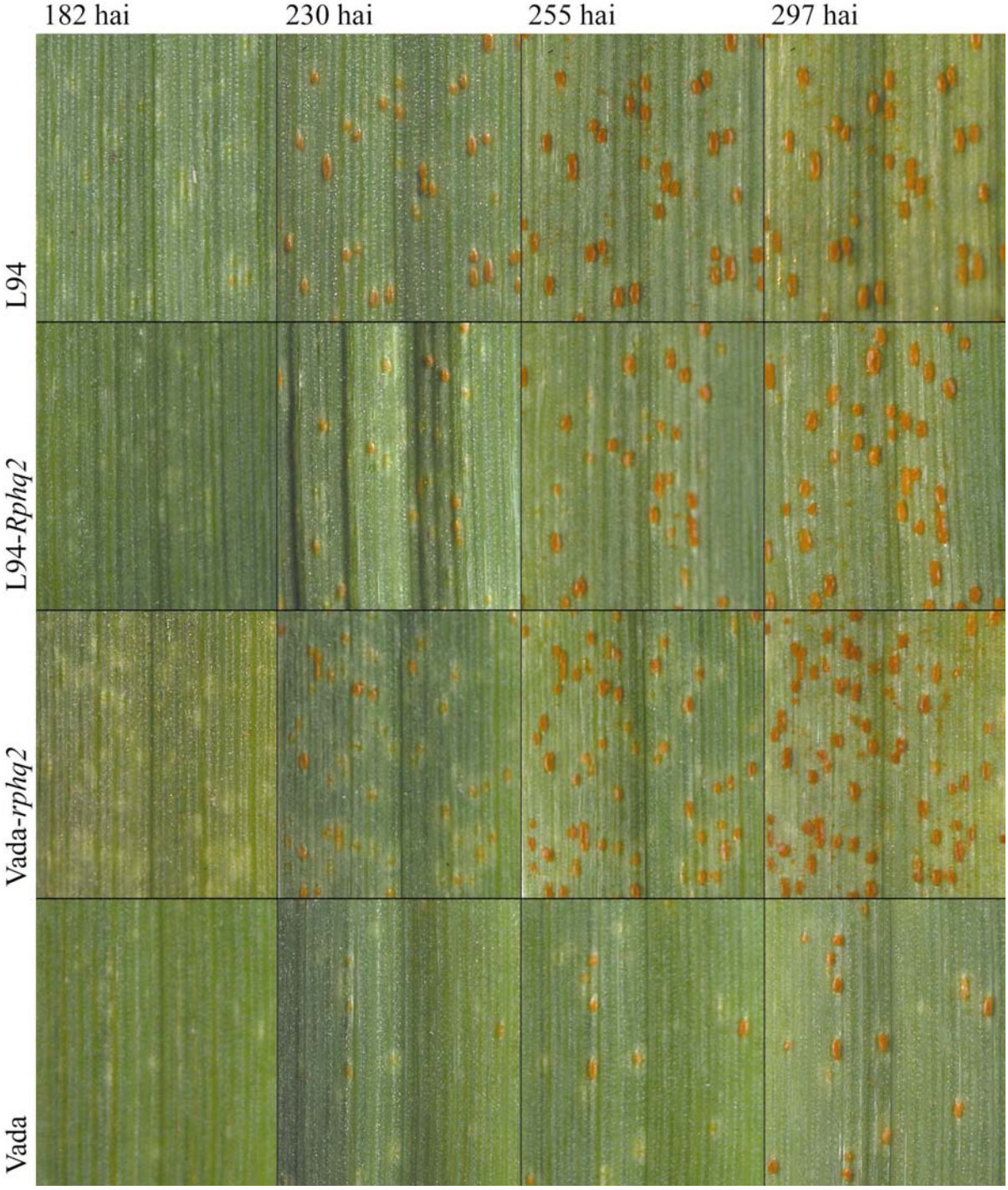

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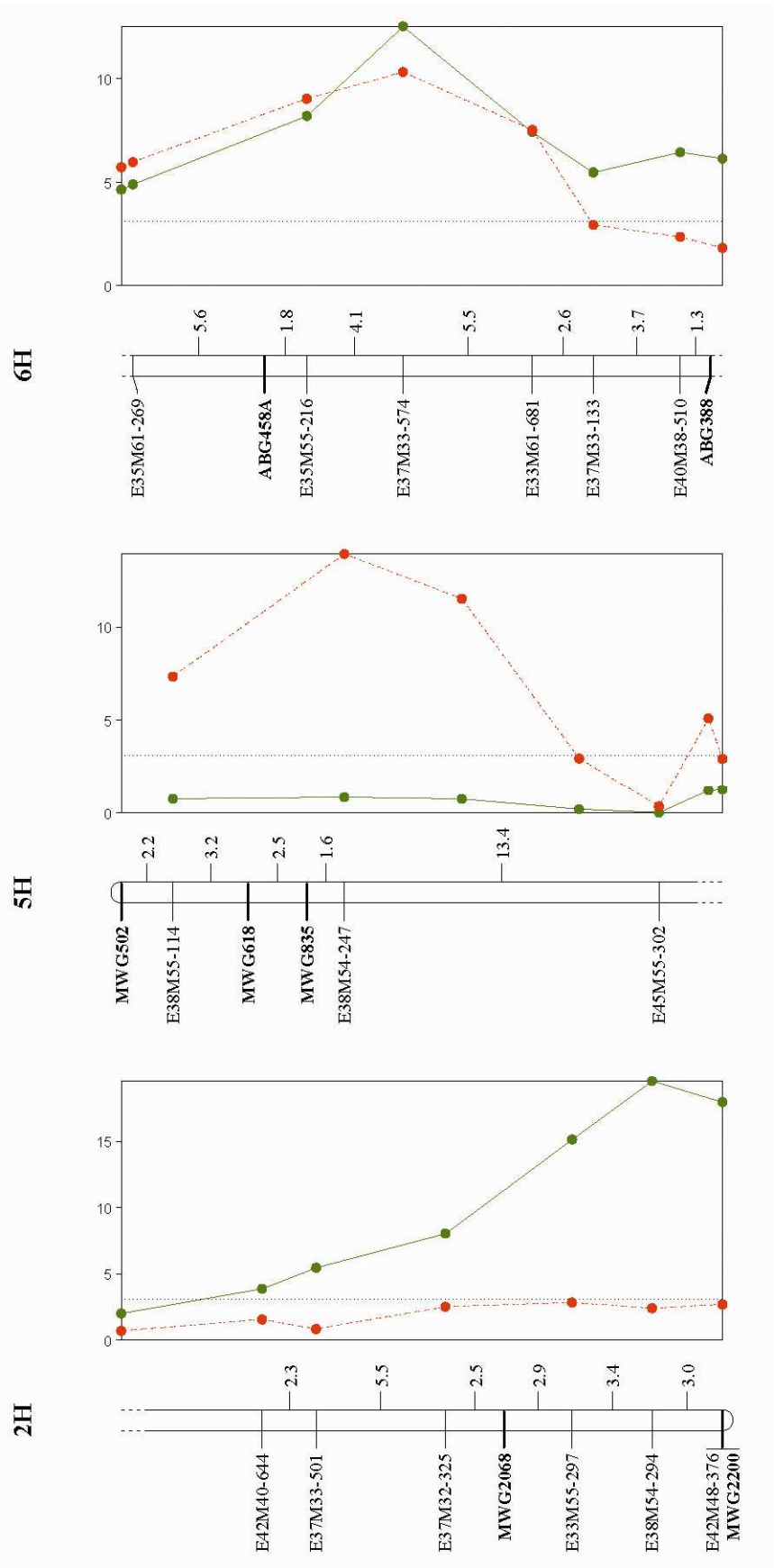

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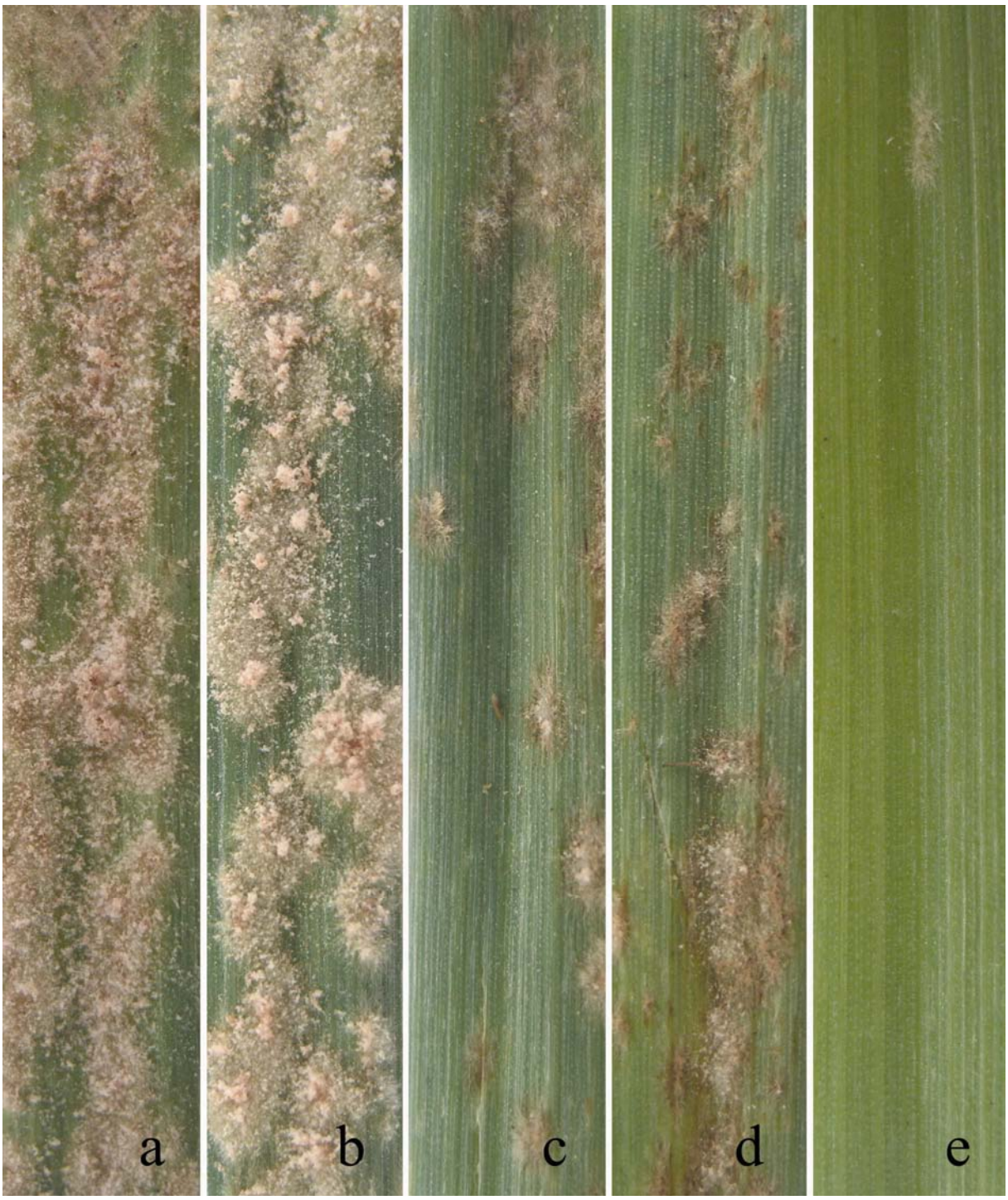

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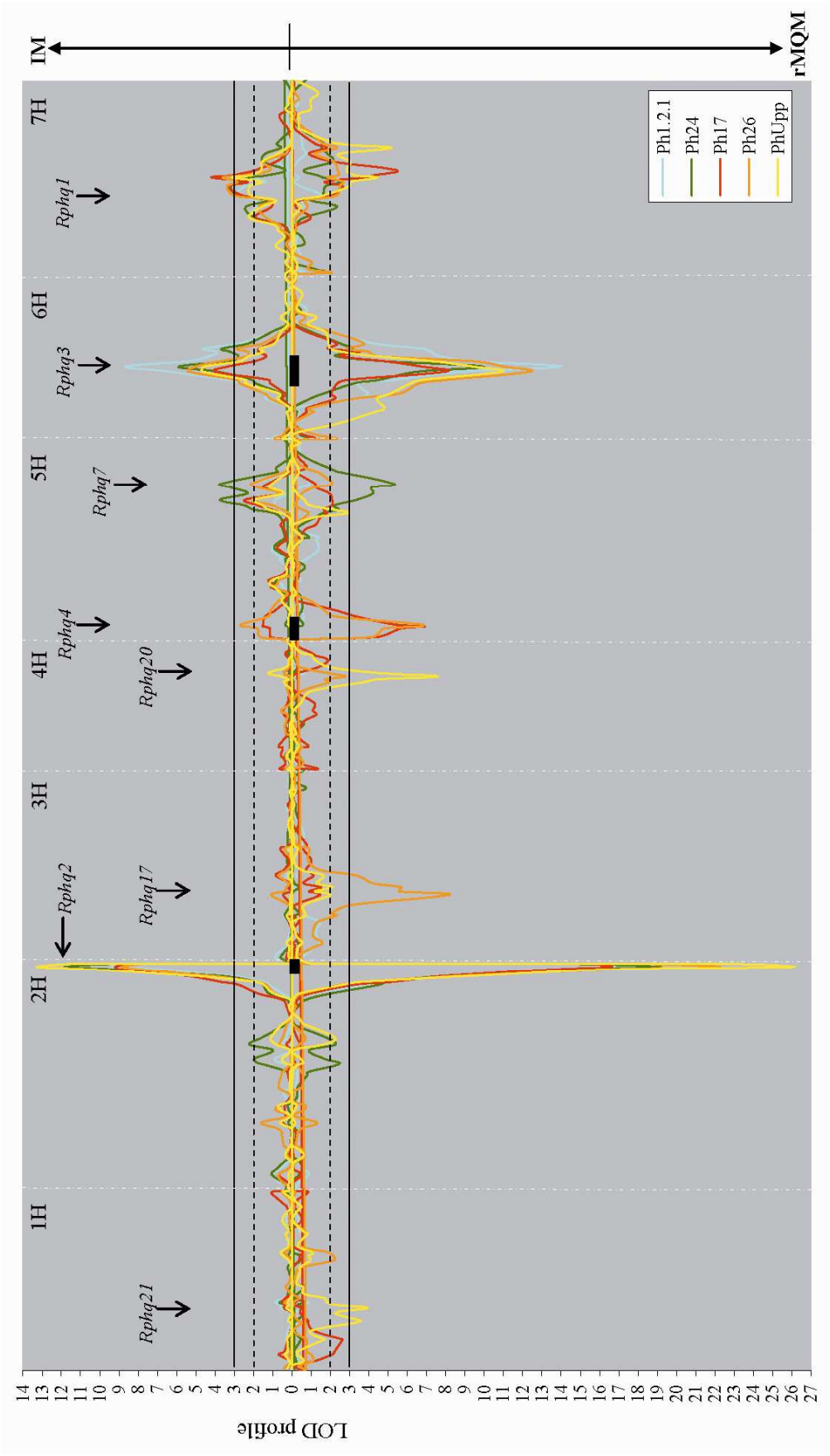

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