Phenotypic assessment and genetic mapping of genes conferring resistance to leaf scald (*Rhynchosporium commune*) in barley (*Hordeum vulgare*)

Kerstin Hofmann

Phenotypic assessment and genetic mapping of genes conferring resistance to leaf scald (*Rhynchosporium commune*) in barley (*Hordeum vulgare*)

Institut für Pflanzenbau und Pflanzenzüchtung I

Justus-Liebig-Universität Gießen

Lehrstuhl für Pflanzenzüchtung

Prof. Dr. Dr. h.c. Wolfgang Friedt

Phenotypic assessment and genetic mapping of genes conferring resistance to leaf scald (*Rhynchosporium commune*) in barley (*Hordeum vulgare*)

Dissertation zur Erlangung des akademischen Grades eines Doktors der Agrarwissenschaften

- Dr. agr. -

Im Fachbereich

Agrarwissenschaften, Ökotrophologie und Umweltmanagement

der Justus-Liebig-Universität Gießen

Vorgelegt von

Kerstin Hofmann

aus Gießen

Gießen im September 2014

Mit Genehmigung des Fachbereichs

Agrarwissenschaften, Ökotrophologie und Umweltmanagement

der Justus-Liebig-Universität Gießen

Prüfungskommission:

1. Gutachter:	Prof. Dr. Dr. h.c. Wolfgang Friedt
2. Gutachter:	Prof. Dr. Karl-Heinz Kogel
Prüfer:	Prof. Dr. Matthias Frisch
Prüfer:	Prof. Dr. Sven Schubert
Vorsitzender:	Prof. Dr. Bernd Honermeier

Tag der Disputation: 14.04.2015

Dedicated to my aunt and uncle

Gertrud and Lothar Schäfer

in loving memory.

So eine Arbeit wird eigentlich nie fertig,

man muss sie für fertig erklären,

wenn man nach Zeit und Umständen das Mögliche getan hat.

Johann Wolfgang von Goethe

Table of contents

Publications

Results presented in this work have been published in advance.

Peer-reviewed article:

Hofmann K, Silvar C, Casas AM, Herz M, Büttner B, Garcia MP, Contraeras-Moreira B, Wallwork H, Igartua E, Schweizer G (2013) Fine mapping of the *Rrs1* resistance locus against scald in two large populations derived from Spanish barley landraces. Theor Appl Genet 126:3091-3102

Contributions to meetings and conferences:

Hofmann K, Silvar C, Casas AM, Herz M, Igartua E, Schweizer G (2010) Fine mapping of the *Rrs1* resistance locus against scald (*Rhynchosporium secalis*) in barley (*Hordeum vulgare*) (poster). GPZ-Tagung “Genomics-based breeding”, 26.-28.10.2010, Gießen: Abstract P19.

Hofmann K, Greif P, Einfeldt C, Holzapfel J, Herz M, Schweizer G (2010) Development of diagnostic markers for pyramiding resistance genes against scald (*Rhynchosporium secalis*) in barley (*Hordeum vulgare*) (poster). 10. GPZ-Haupttagung “Innovations in breeding methodology”, 15.-17.03.2010, Freising: Abstract Session 4/P2 Seite 33.

Hofmann K, Greif P, Einfeldt C, Holzapfel J, Herz M, Schweizer G (2009) *Rhynchosporium secalis* resistance in barley – from mapping to marker development and pre-breeding material (oral presentation). 19th ITMI/3rd COST Tritigen Meeting, 31.08-04.09.2009, Clermont-Ferrand, France: Abstract ITMI2009_016, S. 140.

Hofmann K, Greif P, Einfeldt C, Holzapfel J, Herz M, Schweizer G (2008) Umfassende *Rhynchosporium secalis* Resistenz bei Gerste – von der Kartierung über die Entwicklung diagnostischer Selektionsmarker zum Pre-Breeding Material. 59. Tagung der Pflanzenzüchter und Saatgutkaufleute Österreichs, 25.-27.11.2008, Raumberg/Gumpenstein, Tagungsband S. 39-42.

Hofmann K, Greif P, Einfeldt C, Holzapfel J, Herz M, Schweizer G (2008) Erhöhung des Resistenzpotentials der Gerste (*Hordeum vulgare* L.) gegenüber *Rhynchosporium secalis* durch Nutzung genetischer Diversität. 9. GPZ-Haupttagung „Biodiversität in der Pflanzenproduktion“, 30.09.-02-10.2008, Göttingen: Vorträge für Pflanzenzüchtung Heft 77, ISSN 0934-5116, S. 217-218.

List of figures

List of tables

Abbreviations

AFLP	amplified fragment length polymorphism
BOPA	barley oligonucleotide pool assay
CAPS	cleaved amplified polymorphic sequence
cM	centi morgan
DH	double haploid
DNA	deoxyribonucleic acid
EST	expressed sequence tag
Gb	giga bases
LOD	logarithm of the odds
MAS	marker assisted selection
Mbp	mega base pairs
NIP	necrosis inducing protein
PCR	polymerase chain reaction
QTL	quantitative trait locus
RFLP	restricted fragment length polymorphism
SBCC	Spanish Barley Core Collection
SNP	single nucleotide polymorphism
SSR	simple sequence repeat
STS	sequence tagged site

1 Introduction

1.1 *Hordeum vulgare*

Barley (*Hordeum vulgare* L.) is one of the oldest cultivated crops. Its domestication dates back to 8,000 BC (Zohary and Hopf 2000, Badr et al. 2002). The origin of cultivated barley (*H. vulgare* ssp. *vulgare* L.) is assumed to be the Fertile Crescent, more precisely the Israel/Jordan region, although its wild ancestor, *H. vulgare* ssp. *spontaneum*, can be found in other regions of the world such as North Africa or the Himalayas (Badr et al. 2002).

Nowadays barley is one of the most important crops worldwide and the fourth most important cereal after maize, rice and wheat (Figure 1.1-1).

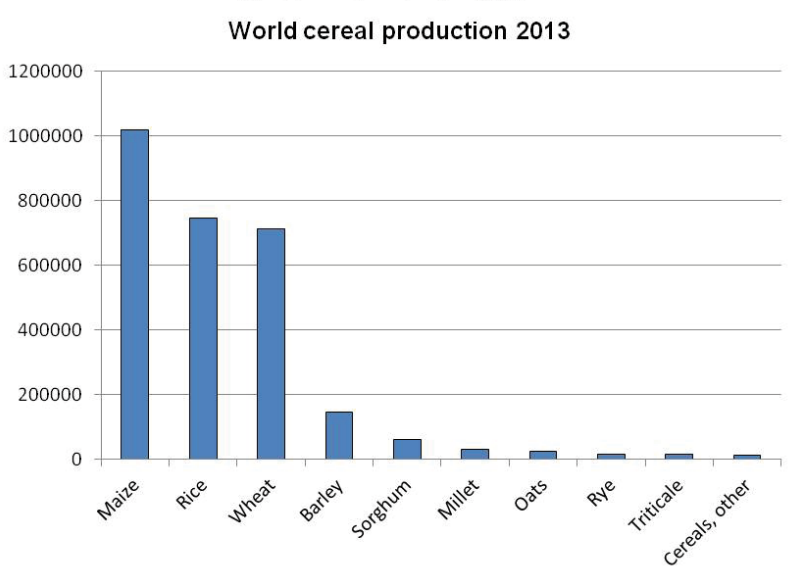

Figure 1.1-1: Worldwide production of cereals (unit: 1000 tons) in 2013 (source: faostat.fao.org).

Barley is mainly produced in the temperate zones of the Northern hemisphere, although other countries like Australia or Argentina contribute considerably to the worldwide production as well (Figure 1.1-2). Germany comes in second place after Russia and before France with a total barley production of approximately 10.3 mio tons in 2013. The overall barley production in Germany rose by 12.4% on average in 2014. The higher increase was observed in winter barley with 13.5%, whereas for spring barley only 7.9% increase was registered (BMEL 2014)

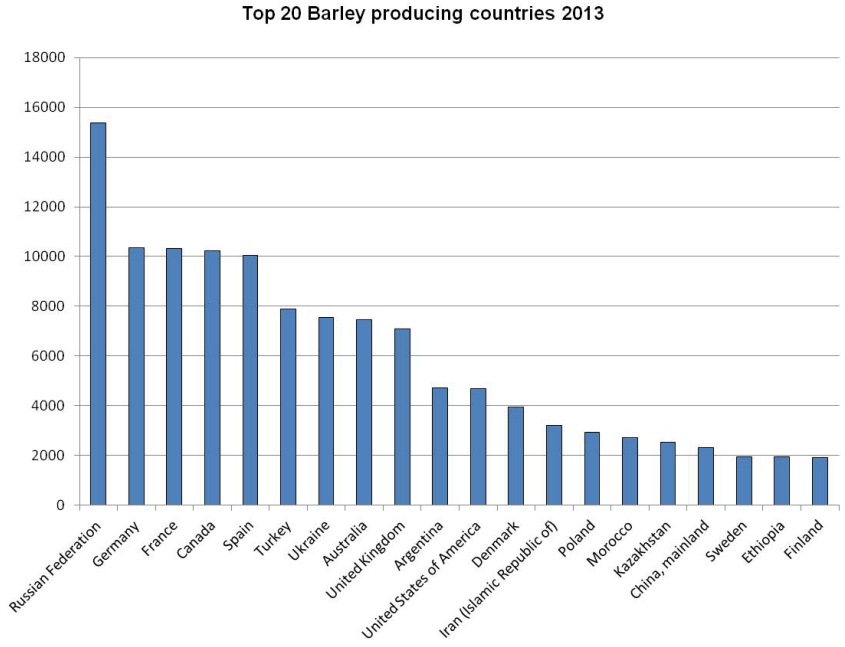

Figure 1.1-2: 20 Biggest barley producing countries worldwide (unit: 1000 tons) in 2013 (source: faostat.fao.org).

Traditionally spring barley is used for malting purposes and characterized by comparatively low yield (20-25% less than winter barley), low protein content (9.5-11.5%) and high malting quality. The main use for winter barley is animal feed, resulting in the major breeding goals high yield and high protein content. More recently breeders have attempted to combine high yield and high malting quality in winter varieties, as well as tolerance to biotic and abiotic stress (Friedt and Ordon 2013).

Barley is an annual diploid plant with 2n = 14 chromosomes and a genome size of about 5.1 Gb (Mayer et al. 2011). It has long been considered as a model organism for plant genetics. Numerous high density consensus maps of the genome have been published using various marker systems (Ramsay et al. 2000, Rostoks et al. 2005, Varshney et al. 2007, Marcel et al. 2007, Stein et al. 2007, Close et al. 2009, Muñoz-Amatriaín et al. 2011). In 2012 a draft of the barley genome was published by the International Barley Sequencing Consortium (IBSC 2012). The physical map of the barley genome can be accessed under mips.helmholtz-muenchen.de/plant/barley/ alongside with the genome zipper aligning the genomes of barley, sorghum, rice and *Brachypodium distychum* in a conserved synteny model (Mayer et al. 2011).

1.2 Scald (*Rhynchosporium commune*)

Scald is one of the major diseases of barley, especially in areas with a cool temperate climate (Zhan et al. 2008). Yield losses of up to 40% have been reported as well as impaired grain quality resulting in lower prices (Shipton et al. 1974). The disease is caused by *R. commune*, a hemibiotrophic haploid fungus belonging to the Ascomycota. The pathogen was first described by Oudemans (1897), who isolated the pathogen from barley and called it *Marsonia secalis*. In the same year Frank (1897) described the fungus as *R. graminicola*. Caldwell (1937) finally established the name *R. secalis* (Oudem) J.J. Davis and assigned the pathogen to the class of Deuteromycetes, and within that class to the order of Moniliales.

Today five species belonging to the genus *Rhynchosporium* are known (Wenzel 2014). What was formerly considered as one species – *R. secalis* – is now split up into three different species with different hosts (Zaffarano et al. 2011). *R. commune* is the elicitor of barley scald, *R. secalis* is able to infect rye and triticale, and various *Agropyron* species belong to the host range of *R. agropyri*. In addition *R. orthosporum* and *R. lolii* infecting *Dactylis glomerata* and *Lolium perenne* respectively are known (Wenzel et al. 2014).

Visible symptoms of scald appear between 5-15 days after inoculation (Ali 1974). Initial symptoms are watery grayish-green spots of about 1-2 cm diameter. Later those spots dry out leaving necrotic light-brown lesions with a darker margin (Figure 1.2-1).

Figure 1.2-1: Typical symptoms of *R. commune* on adult barley leaves (source: LfL Bayern, IPZ1b).

1.3 Scald-barley-interaction

Initial infection with *R. commune* happens through conidia being produced on infected crop debris (Heitefuss et al. 2000, Zahn et al. 2008). The conidia are splash-dispersed by rain fall. After germination on the leaf surface appressoria are formed and the cuticle is penetrated by means of penetration pegs (Zhan et al. 2008). Hyphae spread beneath the cuticle, and after 4-5 days the epidermal cells around the penetration site collapse (Zhan et al. 2008). The hyphae spread further into the intercellular space between epidermis and mesophyll. Approximately two weeks later those cells collapse as well, causing the typical necrotic lesions (Figure 1.3-1, cf. Steiner-Lange et al. 2003).

Histological studies have shown that the collapse of the mesophyll is not caused by physical penetration of the cells (Jorgensen et al. 1993, Xi et al. 2000). Toxic elicitors are the putative reason for the collapse (Wevelsiep et al. 1993). One of these elicitors is the necrosis inducing protein 1 (NIP1) which induces a defense reaction in cultivars carrying the *Rrs1* resistance gene, but a susceptible reaction in cultivars without that gene (Hahn et al. 1993, van't Slot et al. 2003). Those findings suggest a gene-for-gene interaction (Steiner-Lange et al. 2003). Various genes have been described encoding putative virulence factors such as cell wall degrading enzymes in *R. commune* (Steiner-Lange et al. 2003, Siersleben et al. 2014, Wenzel 2014).

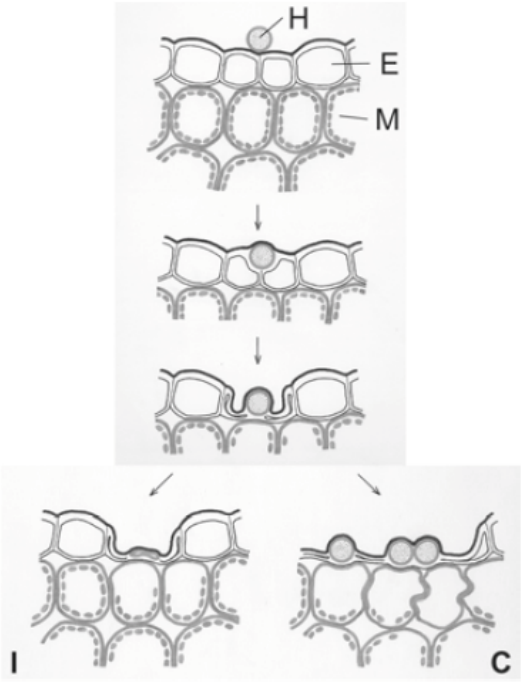

Figure 1.3-1: Early development of scald - “Schematic depiction of the early stages in the development of *Rhynchosporium secalis* on barley leaves. Fungal hyphae (H) have penetrated the leaf cuticle 24 h post inoculation (p.i.). Epidermal cells (E) in the vicinity of fungal collapse 3 to 4 days p.i. Through unknown mechanisms, fungal growth is arrested on resistant plants (incompatible interaction [I]), whereas epidermal collapse continues on susceptible plants (compatible interaction [C]). When the underlying mesophyll cells (M) collapse 2 weeks p.i., necrotic spots become visible. For clarity reasons dimensions of the fungal structures are shown oversized.” (Steiner-Lange et al. 2003)

1.4 Scald resistance in barley

Resistance to scald can be conveyed by single major resistance genes as well as quantitative trait loci (Zhan et al. 2008) which are presumed locations of resistance-associated genes. Major resistance genes have been an object of research since the late 1950s. Schein (1958), Dyck and Schaller (1961), Wells and Skoropad (1963) and later Habgood and Hayes (1971) and Starling et al. (1971) made several attempts to identify the genes underlying observed resistances using differential isolates and phenotypic anchor markers. A number of *Rh*-loci were identified, some describing a different allele of an already known locus. Bjørnstadt et al. (2002) introduced the *Rrs* nomenclature. All confirmed alleles of the *Rh-Rh3-Rh4* complex were named *Rrs1*, with the name of the reference cultivar for the allele attached as a suffix for differentiation. The first genetic map for this locus was published by Barua et al. (1993), Graner and Tekauz (1996) reported co-segregating markers. A high-resolution map of the chromosomal region containing the *Rrs1* resistance locus was published by Hofmann et al. (2013) and is reported in chapter 2. The *Rrs2* locus was mapped as *Rh2* by Schweizer et al. (1995) on the distal part of the short arm of chromosome 7H. To this date *Rrs2* is the only scald resistance gene for which a physical map and diagnostic markers were published (Hanemann et al. 2009). *Rrs3* was assigned to the loci *rh7* and *Rh9* on chromosome 4H found in Abyssinian, Steudelii and Kitchin (Bjørnstadt et al. 2002). Patil et al. (2003) mapped a second resistance gene, *Rrs4*, on chromosome 3H. It mapped on the proximal part of the long arm approximately 20 cM distal from the putative location of *Rrs1*. The last resistance gene to be reported in cultivated barley was $Rrs15_{CIho8288}$. Schweizer et al. (2004) mapped it on the short arm of chromosome 2H in accession CIho8288, and the locus was confirmed by Wagner et al. (2008) in cultivar Triton.

A number of resistance genes were also identified in wild barley accessions and elite barley backcrosses thereof. *Rrs12* was reported by Abbott et al. (1992) on chromosome 7HS, Garvin et al. (2000) located *Rrs14* on chromosome 1HS, and *Rrs15* was mapped on chromosome 7HL by Genger et al. (2005). The overlapping discovery and publication of the resistance genes reported by Genger et al. (2005) and Schweizer et al. (2004) resulted in two different genes carrying the same designation. Zhan et al. (2008) proposed that $Rrs15_{CIho8288}$ by Schweizer et al. (2004) should be renamed *Rrs17* as the gene by Genger et al. (2005) had at that point already been referenced as *Rrs15*. But $Rrs15_{CIho8288}$ was referenced as such in the same year by Wagner et al. (2008) and the proposal from Zhan et al. (2008) has not been picked up since, leaving the scientific community with a confusing nomenclature yet again.

The last new resistance gene against leaf scald was reported by Pickering et al. (2006). The group succeeded in transferring resistance from *Hordeum bulbosum* into *H. vulgare* cv. Emir, and mapped the gene, *Rrs16*, on chromosome 4HS.

The *Rrs13* resistance locus was first reported by Abbot et al. (1991) in a wild barley accession. The same group located the gene on chromosome 6H, with weak linkage to the Dip1-locus (Abbott et al. 1992). Afterwards, Abbott et al. (1995) published a linkage map displaying *Rrs13* on the short arm of chromosome 6H. It was located between RFLP markers Cxp3 and ABG458 with distances of 7.3 and 26.4 cM respectively.

The short arm of chromosome 6H has since repeatedly been associated with scald resistance (Zhan et al. 2008). Several QTL were mapped into this genomic region by Jensen et al. (2002), Cheong et al. (2006) and Shtaya et al. (2006). The latest location of a putative *Rrs13* allele was reported by Wagner et al. (2008). Different markers and marker systems used make it difficult to compare the results from those publications. The precise location of *Rrs13* is still unknown, as are markers closely linked to this locus.

The objective of the present work was to identify the resistance loci present in three barley accessions with Spanish origin, to create high density genetic maps of the chromosome segments containing these loci, to assess their value for breeding, and to develop or identify tightly linked markers for marker assisted selection.

2 Chapter A: Fine mapping of the *Rrs1* resistance locus against scald in two large populations derived from Spanish barley landraces

Kerstin Hofmann, Cristina Silvar, Ana M Casas, Markus Herz, Bianca Büttner, M Pilar Gracia, Bruno Contreras-Moreira, Hugh Wallwork, Ernesto Igartua, Günther Schweizer

K. Hofmann and C. Silvar contributed equally to this publication.

Key message

In two Spanish barley landraces with outstanding resistance to scald, the $Rrs1_{Rh4}$ locus was fine mapped including all known markers used in previous studies and closely linked markers were developed.

Abstract

Scald, caused by *Rhynchosporium commune*, is one of the most prevalent barley diseases worldwide. A search for new resistance sources revealed that Spanish landrace-derived lines SBCC145 and SBCC154 showed outstanding resistance to scald. They were crossed to susceptible cultivar Beatrix to create large DH-mapping populations of 522 and 416 DH lines that were scored for disease resistance in the greenhouse using two *R. commune* isolates. To ascertain the pattern of resistance, parents and reference barley lines with known scald resistance were phenotyped with a panel of differential *R. commune* isolates. Subpopulations were genotyped with the Illumina GoldenGate 1,536 SNP Assay and a large QTL in the centromeric region of chromosome 3H, known to harbour several scald resistance genes and/or alleles, was found in both populations. Five SNP markers closest to the QTL were converted into CAPS markers. These CAPS markers, together with informative SSR markers used in other scald studies, confirmed the presence of the *Rrs1* locus. The panel of differential scald isolates indicated that the allele carried by both donors was $Rrs1_{Rh4}$. The genetic distance between *Rrs1* and its flanking markers was 1.2 cM (11_0010) proximally and 0.9 cM (11_0823) distally, which corresponds to a distance of just below 9 Mbp. The number and nature of scald resistance genes on chromosome 3H are discussed. The effective *Rrs1* allele found and the closely linked markers developed are already useful tools for molecular breeding programs and provide a good step towards the identification of candidate genes.

2.1 Introduction

The fungal disease leaf blotch or leaf scald, caused by the hemibiotrophic haploid fungus *Rhynchosporium commune* (formerly *R. secalis,* Zaffarano et al. 2011), is one of the most prevalent barley diseases worldwide, particularly in the cool and semi-humid barley growing regions (Zhan et al. 2008). Yield losses attributed to this pathogen commonly range around 5–10 %, though losses of up to 40 % have been reported (Paulitz and Steffenson 2011). The fungus and its interaction with barley have been thoroughly reviewed by Zhan et al. (2008) and are described in more detail in Thirugnanasambandam et al. (2011). A comprehensive profile of *R. commune* is shown in Avrova and Knogge (2012).

Barley scald is currently controlled by means of agronomic practices, chemical control and genetic resistance (Avrova and Knogge 2012). In northern Europe, leaf scald is principally controlled by fungicide treatment, although increasing bans on pesticides in the EU reduce the opportunities to achieve good control of pathogens in cereals exclusively through the application of fungicides (Hillocks 2012). The most sustainable and cost-efficient way to reduce the disease is by growing resistant cultivars. The main drawback of both disease management strategies is that *R. commune* is a highly variable pathogen and able to overcome new fungicides and resistances very quickly (Shipton et al. 1974; Xi et al. 2000b; Zhan et al. 2008; Avrova and Knogge 2012). Even in scald-resistant cultivars, the fungus is sometimes able to sporulate to a small extent, without clear symptoms (Ayres and Owen 1971; Thirugnanasambandam et al. 2011; Zhan et al. 2012). Therefore, the demand for resistance genes and/or alleles is still high, as is the need for suitable markers to design more efficient breeding programs and gene pyramiding (Looseley et al. 2012).

The number and nomenclature of scald resistance genes in barley is not settled, though efforts to clarify the different QTLs and genes have simplified the field and in 2002 Bjørnstadt et al. summarized the accepted new *Rrs* classifications (Bjørnstad et al. 2002). Following one of the most recent and thorough reviews on this subject, by Zhan et al. (2008), nine major resistance genes have been identified (designated with an *Rrs* prefix), as well as many QTLs, at least seven of them in genomic locations clearly distinct from the major genes. The sources of resistance are barley varieties (Dyck and Schaller 1961; Habgood and Hayes 1971; Schweizer et al. 1995, 2004; Graner and Tekauz 1996; Cheong et al. 2006; Wagner et al. 2008), landraces (Dyck and Schaller 1961; Garvin et al. 2000; Patil et al. 2003), *Hordeum vulgare* ssp. *spontaneum* (Garvin et al. 2000; Genger et al. 2003; von Korff et al. 2005; Yun et al. 2005) and even one gene from *H. bulbosum* (Pickering et al. 2006).

The *Rrs1* locus was the first to be discovered and has been repeatedly mapped to chromosome 3H (Thomas et al. 1995; Graner and Tekauz 1996; Williams et al. 2001; Grønnerød et al. 2002; Genger et al. 2003) with more than 11 identified alleles (Bjørnstad et al. 2002). However, it is still not clear whether *Rrs1* is a collection of several R-genes close to each other or several alleles of the same gene. In fact, the number and location of scald resistance genes on chromosome 3H of barley is an issue still under debate (Bjørnstad et al. 2002; Wallwork and Grcic 2011). Dyck and Schaller (1961) described two closely linked genes *Rh3* and *Rh4*, which later Habgood and Hayes (1971) described as alleles of the same gene (*Rh* and *Rh4*). Recent studies (Wallwork and Grcic 2011) identified at least two distinct patterns of reaction to scald caused by the gene(s) located close to the centromere on 3H, which are distinguishable using panels of differential isolates and cultivars. Patil et al. (2003) mapped a second resistance locus, named $Rrs4_{CI11549}$, 22 cM distal to *Rrs1* on chromosome 3HL. Finally, several authors have found that dwarfing genes on the long arm of 3H have a pleiotropic effect on scald resistance (Jensen et al. 2002; Looseley et al. 2012).

With the exception of *Rrs2* (Hanemann et al. 2009), there are no diagnostic markers for any of the scald resistance loci. There are markers closely linked to *Rrs1*, namely HVM0027 (SSR), MWG680 (RFLP) and STS_agtc17 (Patil et al. 2003), but they still show recombination with each other and with the resistance gene itself, making them unreliable for precise marker-assisted selection. This is further aggravated by the centromeric region position of *Rrs1* exhibiting very low recombination frequency. Phillips et al. (2010) report that about 20 % of all barley genes may be located in centromeric and subcentromeric regions and thus represent genes with limited accessibility based on genetic mapping approaches. Even if tightly linked markers are used for MAS, linkage drag during introgression of these “centromeric” genes will be large.

To this day, no single publicly available map that integrates all the known SSR and STS markers around the *Rrs1* locus exists. The development of integrated maps and tightly linked markers is recommended to provide more diagnostic markers for scald resistance loci to molecular breeding programs and to analyze genetic haplotypes for association studies. For these reasons, a precise location of “*Rrs1”* in a dense map including as many informative markers as possible is a sensible research objective, especially in terms of combining older SSR with newly developed SNP markers (Illumina) in one map.

Another requisite to improve breeding for disease resistance is the identification of additional resistance sources, preferably in the primary genepool of *H. vulgare*. Collections of landraces represent valuable resources containing broad genetic variability for numerous agronomically important traits. One of these collections is the Spanish Barley Core Collection (SBCC), a representative sample of the landraces traditionally cultivated in Spain, comprising 175 genetically diverse genotypes (Igartua et al. 1998; Lasa 2008). The SBCC was evaluated for resistance against multiple pathogens and a remarkably high number of lines (26 %) presented good resistance to scald (Silvar et al. 2010). Two of the highly resistant lines, SBCC145 and SBCC154, were chosen for further investigation and fine mapping of the scald resistance loci in two large DH populations.

So, the objectives of the present work were to (1) identify the factors underlying the resistance to scald of both SBCC lines, (2) locate them in a dense map with publicly available markers as well as all known markers used in previous *Rrs1* studies and (3) develop tightly linked markers suitable for the rapid incorporation of these loci in barley breeding programs.

2.2 Material & Methods

2.2.1 Plant material and fungal isolates

The lines SBCC145 and SBCC154, from the SBCC, were selected for their outstanding resistance to *R. commune* (Silvar et al. 2010). SBCC145 is a six-rowed intermediate barley line (with a mild vernalization requirement) and is also resistant to powdery mildew (Silvar et al. 2011), whereas SBCC154 is a two-rowed facultative barley. Both lines were crossed with Beatrix (Viskosa/Pasadena), a cultivar from the German breeder Nordsaat. Beatrix is a two-rowed spring barley with good malting quality and is highly susceptible to scald.

For scald resistance and further mapping studies, two doubled haploid (DH) populations consisting of 522 (SBCC145 × Beatrix) and 416 DH lines (SBCC154 × Beatrix) were generated by anther culture from the F1 generation. From them, subpopulations of 190 and 168 DH lines, respectively, were created for whole genome genotyping and QTL analysis. Lines for each subpopulation were selected randomly based on the expression of a clear phenotype in response to scald infection. In addition to the susceptible parent Beatrix, the two-rowed German spring barley varieties, Steffi (Saatzucht Ackermann, Irlbach) and Alexis (Saatzucht Breun, Herzogenaurach), were used as scald-susceptible references for phenotyping. A set of 11 additional barley accessions with one or two known resistance loci was used for comparison purposes on the level of resistance of SBCC145 and SBCC154 (Table 2.3-1).

Five genetically diverse isolates of *R. commune* (S147-1, Rhy17, Rhy174, UK7, LfL07) from the collection held at the Bavarian State Research Center for Agriculture in Freising (Germany) were used to assess the level of resistance of Spanish lines in comparison with the set of 11 barley accessions. Isolate 271 was used to phenotype the entire DH populations and isolate LfL07 was used for additional phenotyping of the subpopulations used for whole genome genotyping. Isolates 271 and LfL07 produced similar reactions on the parents of both populations. The *R. commune* isolates were cultivated in liquid media in the dark at 15 °C. The spore suspension was produced by planting pea-sized pieces of mycelium on lima bean agar to induce the formation of spores. Spores were harvested after 2 weeks of growth, at 15 °C in the dark, by scraping culture plates with 5 ml RO water and then diluted to a concentration of 2 × 105 spores/ml (Hanemann et al. 2009).

Two additional isolates of *R. commune*, 332a and 385 from the collection held at the South Australian Research and Development Institute (SARDI) in Adelaide were tested in Australia against the Spanish parent lines and 4 of the 11 barley accessions. These two isolates are able to discriminate between specific virulence patterns of *Rrs1* alleles found in the centromeric region of chromosome 3H (Wallwork and Grcic 2011). At SARDI, the two isolates were cultivated following the procedures detailed in Wallwork and Grcic (2011) using an end concentration of 1 × 106 spores/ml for inoculation.

2.2.2 Resistance assessment

At Freising, an assessment of resistance to *R. commune* for the parents, checks and the two populations was carried out according to Schweizer et al. (2004) and Hanemann et al. (2009) with two isolates (271 and LfL07). Each trial consisted of four individual plants of each DH line. Plants were grown in the greenhouse at 18 °C for 3 weeks to the early three-leaf stage and then sprayed with a spore suspension adjusted to a concentration of 200,000 spores/ml. Immediately after spray inoculation, the plants were maintained in the dark with 100 % humidity for 48 h. Approximately at 14–21 days after inoculation, scald symptoms were rated on the second leaf of each individual plant three times every other day according to Jackson and Webster (1976), using half steps for better discrimination. The data presented in this work (Figure 2.3-1) are the average score of all four plants per DH line and isolate. The German varieties Steffi and Alexis were used as scald-susceptible reference cultivars. At SARDI/Adelaide, the tests were also conducted under controlled environmental conditions, following the procedures explained in detail in Wallwork and Grcic (2011). Merging the data of both scales leads to *R* = 0, *R*/MR = 1, MRMS = 2, *S*/MS = 3 and *S* = 4.

2.2.3 Genotyping

Genomic DNA was isolated from frozen barley leaves by using the NucleoSpin Plant II Minikit (Macherey–Nagel, Düren, Germany) or according to Behn et al. (2004). To check for the association between resistance and markers previously linked to scald resistance in other populations, the populations were genotyped with HVM0027 (Ramsay et al. 2000) and STS_agtc17, for *Rrs1* (Grønnerød et al. 2002; Patil et al. 2003) and

with HVM0060 for *Rrs4* (Patil et al. 2003). To identify polymorphic markers surrounding the known *Rrs1* locus, SBCC145, SBCC154 and Beatrix were screened with all available SSRs (Ramsay et al. 2000; Li et al. 2003; Rostoks et al. 2005; Stein et al. 2007; Varshney et al. 2007) and SNPs (Rostoks et al. 2005; Stein et al. 2007) in the centromeric region of chromosome 3H. Markers for *Rrs15* (GemS13; Schweizer et al. 2004) and *Rrs2* (Atlas14, AcriCaps; Hanemann et al. 2009) were not polymorphic in the present populations.

QTL mapping was conducted to check for scald resistance loci over the whole genome. With this purpose, both subpopulations of 190 and 168 DH lines described above were genotyped with the 1,536-SNP Illumina GoldenGate Oligonucleotid Pool Assay (Barley OPA1 or BOPA1) (Close et al. 2009) as described previously for SBCC145 × Beatrix (Silvar et al. 2011). Five BOPA1 markers (11_0010, 11_0205, 11_0315, 11_0823 and 11_1476) that were closely linked to the resistance QTL (most likely *Rrs1*) were converted into CAPS markers (Table 2.6-1) and used to genotype the entire populations. These markers were positioned in the Genome Zipper (Mayer et al. 2011) and in the barley physical map to realize the size of the genomic region of interest. Their position was determined by running BLASTN (Altschul et al. 1997) with options—task megablast-dust no, against all anchored contigs and genes (datasets AC1, AC2 and AC3) of the barley physical map (IBSC 2012), available from ftp://ftpmips.helmholtz-muenchen.de/plants/barley/public_data/.

2.2.4 Linkage and QTL analysis

Single and integrated linkage maps were constructed with JoinMap 4.0 (van Ooijen 2006), using Kosambi's map function (Kosambi 1944) and a minimum logarithm of the odds ratio (LOD score) of 5. A map with just BOPA1 markers for the subpopulation SBCC145 × Beatrix was previously constructed for all seven chromosomes (Silvar et al. 2011). For chromosome 3H, a detailed consensus map comprising all SSRs and the five closest BOPA markers was built using the information from the entire populations.

QTL analysis was performed using MapQTL 5.0 (van Ooijen 2004). The interval mapping (IM) procedure was used in a preliminary analysis to identify major QTLs and to detect significantly associated markers. These markers were then used as cofactors in a multiple QTL model (MQM) (Jansen and Stam 1994). MQM was repeated iteratively

by adding significant 'peak markers' at each step as cofactors, until a stable LOD profile was reached. The LOD threshold for detecting QTLs was calculated by a permutation test with 1,000 iterations and a genome-wide significance level of 0.05. This procedure was followed for the whole genome scans of the subpopulations genotyped with BOPA1, and also for chromosome 3H for the whole populations. The interaction between QTLs was analyzed by means of analysis of variance, including as sources of variation the closest markers to the QTL peaks, and calculated using the unbalanced anova routine implemented in Genstat 14 (VSN International 2011). *Rrs1* was also mapped as a binary trait, assigning a score of 0 (resistant) to lines with average disease resistance scores between 0 and 1.9, and a score of 1 to lines with disease scores between 2.0 and 4.0.

2.3 Results

2.3.1 Disease resistance

In a first step, the scald-resistant lines SBCC145 and SBCC154 out of the Spanish Barley Core Collection were phenotyped for disease resistance with five different *R. commune* isolates (Table 2.3-1) along with another 11 reference donors for scald resistance (*Rrs1*$_{Rh4}$, *Rrs2, Rrs15, Rrs1, Rrs13*) and three highly susceptible cultivars (Alexis, Barke, Steffi).

All resistance donors showed mild disease symptoms to at least one *R. commune* isolate in the form of small isolated necrosis on the leaf surface or leaf margins after infection (no large lesions), whereas cultivar Pewter, Atlas, Escaldadura15 and CIho2235 showed weak symptoms with all isolates. Mean infection scores of the resistant accessions with these isolates ranged from 0.0 to 0.9. SBCC145 and SBCC154 had some of the lowest scores, with means of 0.2 and 0.2, respectively. Only the accessions with more than one resistance locus (Atlas46, Osiris, PI 452395, CIho3515, Triton) showed comparable infection scores (0.1–0.2). Accessions with only one resistance locus and without *Rrs1*$_{Rh4}$ like Atlas, CIho2235, CIho8288, Pewter and WW Glabron on the other hand were less resistant (0.4–0.9). The susceptible cultivars reached mean infection scores of 4.0 (Table 2.3-1). An additional independent phenotyping of the landraces, SBCC145 and SBCC154, and reference lines with the differential scald isolates, 332a and 385, at SARDI revealed distinct patterns of resistance (Table 2.3-1), by which all resistant lines with the assumed *Rrs1*$_{Rh4}$ locus (SBCC145, SBCC154, Osiris and CIho3515) were resistant to isolate 332a and susceptible to isolate 385 (Wallwork and Grcic 2011). In case of CIho3515, the R-gene *Rrs13* improved the infection score.

Table 2.3-1: Disease reactions of 15 barley accessions, landraces and cultivars against up to seven isolates of *R. commune* (formerly *R. secalis*). Cultivars Alexis, Beatrix and Steffi were included as susceptible controls.

Barley lines (references)	*Rhynchosporium commune* isolates[a]								R-gene(s) (according to literature)
	S147-1	Rhy 017	Rhy 174	UK7	LfL07	Mean S147-1 - LfL07	332a	385	
SBCC145[c]	0.0	0.0	0.5	0.5	0.0	0.2	0.0	4.0	*Rrs1* $_{Rh4}$
SBCC154[c]	0.0	0.0	0.6	0.6	0.0	0.2	0.0	4.0	*Rrs1* $_{Rh4}$
Atlas[d,e,f,l]	0.1	0.3	0.8[b]	0.8	0.9[b]	0.6	4.0	4.0	*Rrs2*
CIho2235[g,l]	0.3	0.2	0.4	0.2	1.1	0.4	–	–	*Rrs2*
Pewter[g,l]	0.9	0.8	1.2	0.6	1.3	0.9	–	–	*Rrs2*
Escald. 15[g,l]	0.3	0.1	0.7[b]	1.3	–	0.6	–	–	*Rrs2*
CIho8288[j,k]	1.3	0.0	0.6	0.5	1.2	0.7	–	–	*Rrs15*
Atlas 46[e,f,g,l]	0.4	0.3	0.0	0.0	0.0	0.1	4.0	0.0	*Rrs1 + Rrs2*
Osiris[e,f,g,l)]	0.0	0.0	0.4	0.3	0.0	0.1	0.0	3.5	*Rrs1* $_{Rh4}$ *+ Rrs2*
PI 452395[g,l]	0.5	0.2	0.0	0.1	0.0	0.2	–	–	*Rrs1 + Rrs2*
CIho3515[f,g,h]	0.0	0.0	0.4	0.3	0.1	0.2	0.0	1.0	*Rrs1* $_{Rh4}$ *+ Rrs13*
Triton[k]	0.1	0.0	0.0	0.0	0.0	0.0	–	–	*Rrs1 + Rrs15*
Alexis	4.0	4.0	4.0	3.9	4.0	4.0	–	–	Susceptible
Beatrix	4.0	4.0	3.9	4.0	4.0	4.0	–	–	Susceptible
Steffi	4.0	4.0	4.0	4.0[b]	4.0	4.0	–	–	Susceptible

–, not determined

[a]Isolates S147-1 - LfL07 were tested at LfL in Freising in the greenhouse at early three-leaf stage with four seeds/accession in two replications, as described in "Materials and methods". In addition, to discriminate the *Rrs1* locus with differential scald isolates 332a and 385 for the *Rrs1* locus (Wallwork and Grcic *2011*), the SBCC lines 145 and 154, as well as four reference lines, were tested at SARDI following the procedures explained in "Materials and methods"

[b]Plants were tested in a single greenhouse test with four plants

References: [c]This publication, [d]Dyck and Schaller (*1961*), [e]Schweizer et al. (*1995*), [f]Wallwork and Grcic (*2011*), [g]Schweizer et al. (unpublished), [h]Habgood and Hayes (*1971*), [i]Bjørnstad et al. (*2002*), [j]Schweizer et al. (*2004*), [k]Wagner et al. (*2008*), [l]Hanemann et al. (*2009*)

The whole DH-mapping populations SBCC145 × Beatrix (*n* = 522) and SBCC154 × Beatrix (*n* = 416) were phenotyped for scald resistance with the *R. commune* isolate 271 (Fig. 1). Infection scores for the parental lines were on average 0.0 for SBCC145, 0.0 for SBCC154 and 4.0 for Beatrix. Mean infection scores for the populations were 2.63 and 2.32 for SBCC145 × Beatrix and SBCC154 × Beatrix, respectively. The segregation ratios were 215 (*R*): 305 (*S*) for SBCC145 × Beatrix and 149 (*R*): 264 (*S*) for SBCC154 × Beatrix, with 0–1.9 considered resistant (*R*) and 2.0–4.0 being susceptible (*S*) in both cases. Deviation from the expected 1:1 segregation was significant (χ^2 test) at 0.01 for both populations (χ^2 = 15.58 for SBCC145 × Beatrix, χ^2 = 32.02 for SBCC154 × Beatrix). The bimodality observed points to the presence of one large QTL, although the deviation from a 1:1 segregation might suggest the presence of additional minor QTLs (Figure 2.3-1). To account for this, two randomly selected smaller subpopulations from SBCC145 × Beatrix (*n* = 190) and SBCC154 × Beatrix (*n* = 168) were used (“Materials and methods”) to perform whole genome QTL analysis with the Illumina Barley OPA1 chip. In addition to the disease scoring with *R. commune* isolate 271, the subpopulations were additionally phenotyped with the scald isolate LfL07, and the infection scores for the parental lines were 0.0 for SBCC145, 0.0 for SBCC154 and 4.0 for Beatrix. Mean infection scores for the subpopulations were 2.5 and 2.4 for SBCC145 × Beatrix and SBCC154 × Beatrix, respectively. Segregation ratios were 83 (*R*): 107 (*S*) and 62 (*R*): 80 (*S*) for SBCC145 × Beatrix and SBCC154 × Beatrix, respectively (Figure 2.6-1). Segregation did not deviate from 1:1 at a level of 0.01 for both subpopulations (χ^2 = 3.03 for SBCC145 × Beatrix and χ^2 = 2.28 for SBCC154 × Beatrix).

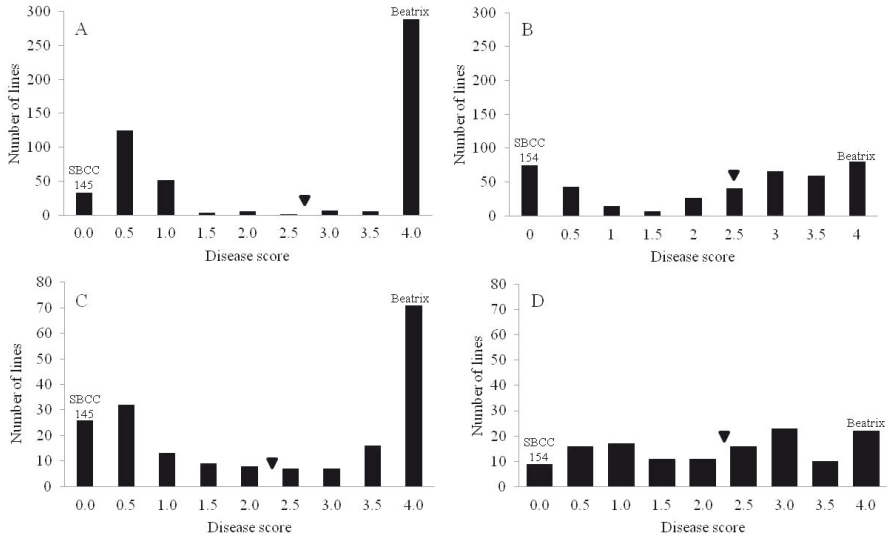

Figure 2.3-1: Response of SBCC145 × Beatrix and SBCC154 × Beatrix DH lines to *R. commune* isolates 271 and LfL07. The Jackson and Webster (1976) scale extended by half steps was used. Vertical arrows indicate mean disease scores. a, b Both DH populations (n = 522/SBCC145 × Beatrix, n = 416 SBCC154 × Beatrix) were tested with isolate 271. c, d Isolate LfL07 was used with those subpopulations genotyped with BOPA1 (n = 190/SBCC145 × Beatrix, n = 168/SBCC154 × Beatrix).

2.3.2 Mapping of the scald resistance locus

Both mapping populations were genotyped with markers linked to previously published scald resistance genes and, as a preliminary result, the main scald resistance locus in both populations was linked to markers HVM0027 and STS_agtc17, close to the centromeric region of chromosome 3H. This location suggested that the *Rrs1* locus was the major factor underlying the resistance of both SBCC lines. Consequently, publicly available markers, including all known markers used in previous studies to locate the *Rrs1* locus, and those close to the centromeric region of chromosome 3H were screened for polymorphism between the parental lines and used for map construction (Table 2.6-2).

In a second step, a whole genome mapping of the SBCC145 × Beatrix subpopulation was performed with Illumina-BOPA1 as reported in Silvar et al. (2011). Regarding the SBCC154 × Beatrix subpopulation, 636 out of 1,536 SNPs were polymorphic between SBCC154 and Beatrix and were used for the construction of the linkage map, which comprised a total length of 1,256.9 cM (Figure 2.6-2). QTL analyses were then carried out for both SBCC145 × Beatrix and SBCC154 × Beatrix subpopulations. A major QTL was detected in both subpopulations for both isolates, with LOD scores between 37.8 and 141.3 on chromosome 3H, close to the centromeric region, and in accordance with the preliminary marker results (Figure 2.6-3, Table 2.3-2).

Table 2.3-2: Summary of QTLs for scald resistance detected in the SBCC145 × Beatrix and SBCC154 × Beatrix DH populations in response to inoculation with *R. commune* isolates 271 and LfL07.

Population	QTL No.	Linkage group	QTL (cM)	Interval (cM)	Closest marker	LOD score	R^2 (%)	Additive effect
SBCC145 × Beatrix	Isolate 271							
	1	2H	31.4	30.3–33.4	11_1175	3.6	0.5	−0.12
	2	3H	70.4	70.0–72.1	11_0205	141.3	96.2	−1.68
	3	4H	53.1	52.5–53.9	11_1316	10.6	1.2	−0.88
	Isolate LfL07							
	1	3H	70.0	68.0–70.8	11_0205	73.4	84.3	−1.52
SBCC154 × Beatrix	Isolate 271							
	1	2H	37.1	35.1–37.5	11_1159	2.8	1.5	−0.19
	2	3H	70.3	72.0–73.1	11_0010	61.5	88.3	−1.53
	Isolate LfL07							
	1	3H	72.5	72.0–73.1	11_0010	37.8	73.1	−1.14

The columns QTL and Interval show the position of the peak and the 2-LOD confidence intervals. R^2 is the percentage of phenotypic variance explained by the QTL. A negative value for the additive effect indicates that the allele from SBCC145 or SBCC154 reduced the value of the trait

Two additional minor QTLs were detected for isolate 271 on chromosomes 2H and 4H. The first one was present in both subpopulations, with LOD scores of 3.6 (SBCC145 × Beatrix) and 2.8 (SBCC154 × Beatrix) (Table 2.3-2). The second minor QTL on chromosome 4H was detected only in the SBCC145 × Beatrix subpopulation, with an LOD score of 10.6 (Table 2.3-2). There was no significant interaction among QTLs in the population SBCC154 × Beatrix. However, in SBCC145 × Beatrix, a significant three-way interaction between the three QTLs was detected for resistance to isolate 271. Also, the interactions of the two minor QTLs with the large QTL on 3H were close to the significance threshold (*P* values of 0.05 and 0.07). This was caused by the more conspicuous effect of the minor QTL in the presence of the resistant allele at the 3H large QTL (Table 2.3-3).

Table 2.3-3: Mean scald resistance values against *R. commune* isolate 271 in the subpopulation SBCC145 × Beatrix for the DH lines grouped according to the haplotypes presented at the markers closest to the QTL peaks.

Haplotype[a]	n	Scald resistance scores[b]	
aaa	15	0.33	a
aba	12	0.39	ab
baa	27	0.58	b
bba	19	1.00	c
aab	25	3.68	d
abb	15	4.00	e
bab	34	3.97	e
bbb	35	3.98	e

[a]Haplotypes for alleles at markers 11_1175 (2H), 11_1316 (4H) and 11_0205 (3H); a SBCC145 allele; b Beatrix allele

[b]Means followed by the same letter are not significantly different (LSD, P < 0.05)

Using the genotypic information of both subpopulations, an integrated map of chromosome 3H was constructed. Five BOPA1 markers that mapped closest to the resistance locus (11_1476, 11_0010, 11_0823, 11_0205 and 11_0315) were converted into CAPS markers (Table 2.6-1) and mapped in the population SBCC145 × Beatrix (*n* = 522) and SBCC154 × Beatrix (*n* = 350) in which they were polymorphic (all five in SBCC154 × Beatrix, all but 11_0010 and 11_0823 in SBCC145 × Beatrix). A high confidence consensus map of chromosome 3H using the information of all lines of both populations was constructed (Figure 2.3-2). It covered a region of 49.1 cM around the resistance

locus. Four BOPA markers (11_0010, 11_0823, 11_0205, 11_0315) mapped into the gap between *Rrs1* and the closest SSR (Bmag0006) or STS (Falcon and STSagtc17) markers, whereas SNP 11_1476 mapped together with the closest proximal SSR marker GBM1242. The genetic distance in the consensus map of the interval comprising the resistance locus was 2.1 cM.

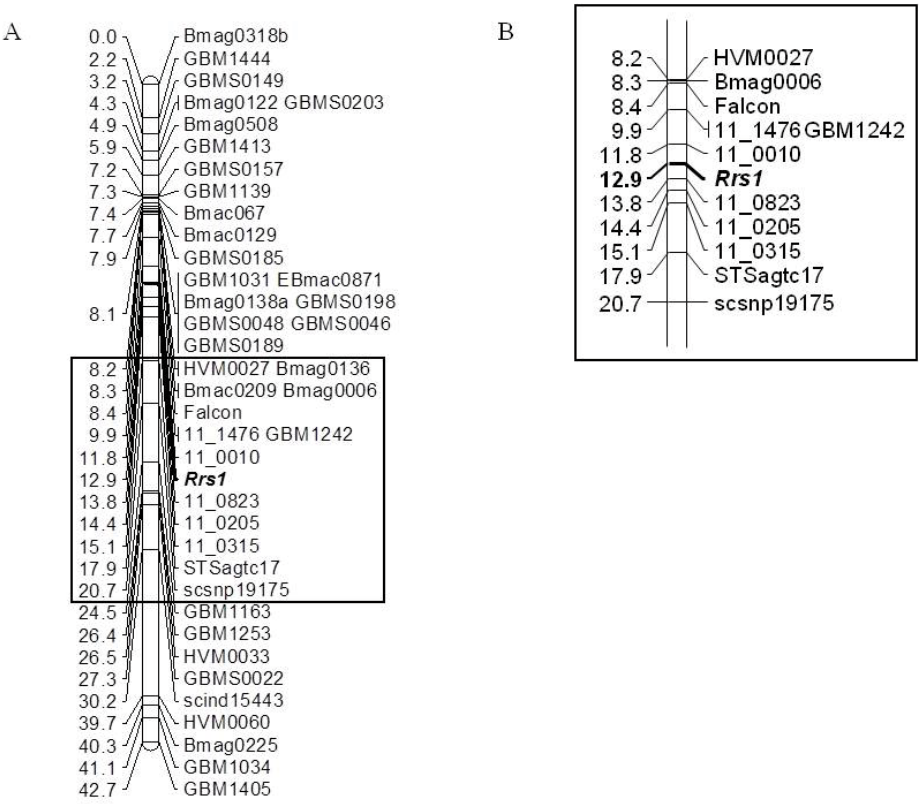

Figure 2.3-2: A: Integrated map of the QTL region of chromosome 3H derived from 872 DH lines coming from the SBCC145 × Beatrix and SBCC154 × Beatrix populations. The map covers the region of the *Rrs1* locus. *Rrs1* itself was mapped as a binary trait. B: Close-up view of the region around *Rrs1*.

In the Genome Zipper (Mayer et al. 2011), the region encompassing markers 11_0010 and 11_0823 comprised a modest number of 16, 12 and 12 genes of *Brachypodium*, rice and sorghum, respectively. Including 11_0315 as the safe lower flanking marker, as present in the two populations, increased the number of syntenic genes in the Genome Zipper to 26, 22 and 23, respectively, for the three species.

The CAPS markers were placed in the physical map of barley. Sequences corresponding to genetic markers 11_0010 (1_0005) and 11_0823 (1_0728) were obtained from Close et al. (2009). The matched contig for 11_0010 had coordinate 377656880 in Morex, Bowman and Barke physical maps, which corresponds to a genetic distance of 53.26 cM in chromosome 3H. However, marker 11_0823 was assigned to two different, but close, positions: 383952360 in Morex and Bowman, and 386536520 in Barke (54.21 cM and 54.53 cM, respectively). We decided to take the largest (8.9 Mb) defined interval to identify anchored genes within it. Although the barley physical map still does not resolve the fine order of genes, a total of 30 high confidence genes were found, and their annotations retrieved from the file barley_HighConf_genes_MIPS_23Mar12_HumReadDesc.txt. CAPS marker 1_0158 (11_0205) was not located in Genome Zipper or in the physical map. Marker 11_0315 (1_0281) corresponded to position 55.15 cM, 389321560 bp in Bowman and Barke physical maps, and may be used as a safe external flanking position for further fine mapping.

2.4 Discussion of chapter A

Previous screening of the Spanish Barley Core Collection revealed that several landrace-derived lines were highly resistant to the *R. commune* isolate Sachs 147-1 (Lasa 2008; Silvar et al. 2010). Two of the most resistant lines, SBCC145 and SBCC154, were selected to further investigate the genetic basis underlying their outstanding resistance to scald using two large DH populations from crosses with the susceptible cultivar Beatrix. The goal was the development of tightly linked markers for selective incorporation of these loci in barley breeding programs.

An extensive screening of both populations with publicly available markers revealed that the resistance locus in both populations co-located with the *Rrs1* resistance gene on 3HL, close to the centromeric region. The distorted ratios of segregation for resistant and susceptible plants were probably caused by an underlying distortion of allelic frequencies in the region of the main QTL in 3H, and not by the presence of more than one major QTL. All SNPs analyzed in the area surrounding this QTL presented distorted allelic frequencies in both populations, between 0.34 and 0.40 for the SBCC145 allele and between 0.31 and 0.39 for the SBCC154 allele, in a region encompassing 20 and 40 cM, respectively (Table 2.6-3). Such deviations are not unusual for DH populations derived from anther culture (Graner and Tekauz 1996; Sayed et al. 2002). Therefore, it is safe to assume that this major resistance gene locus was the main cause for the outstanding resistance in both populations, as shown for the CAPS marker 11_0205 in Figure 2.6-1A, showing a distorted segregation at a ratio of about 40 % resistant to 60 % susceptible lines in both populations.

Besides the main QTL on 3H at the *Rrs1* locus, two minor QTLs were found in this work. A review of common markers across several maps suggests that the two minor QTLs found in this work may have been detected by other studies. The two markers closest to the QTLs on 2H (Table 2.3-2) might be marking the same region, even though they are 11 and 15 cM apart in each population. These QTL also lie within the region where *Rrs15* was detected by Schweizer et al. (2004). In that same region, von Korff et al. (2005) and Wagner et al. (2008) found QTL for scald resistance coming from wild and cultivated barley, respectively. The *Rrs15* locus was further checked with linked markers for their influence on scald disease in both populations. The results confirmed the region, but did not point to the presence of a functional allele for the $Rrs15_{8288}$ major resistance gene (data not shown). The second QTL on chromosome 4H could mark the position of a QTL detected by von Korff et al. (2005), flanked by HVM0013 and GMS0089 on bin 5 of 4H.

The presence of an interaction effect between the three loci detected in SBCC145 × Beatrix and isolate 271 could be caused by a mathematical artefact. Three of the haplotypes shown in Table 2.3-3, *abb*, *bab* and *bbb* present mean disease scores very close or equal to the maximum (4.0). Only when the "resistant" alleles for two minor QTL were present at the same time was their combined effect noticeable. It is possible that we were not able to discriminate visually among plants belonging to these classes, all of them highly susceptible, presenting very large areas of infected leaves. At the other end of the spectrum, for the most resistant plants (i.e. plants with a "resistant" allele at the 3H QTL), it was easier to discriminate between different degrees of attack. For example, it was easy to distinguish between plants covered with scald on 1 % of the surface vs. plants covered by scald on 5 % of the surface. But it was very difficult to differentiate between leaves that were covered by disease spots on 81 or 85 % of the surface. In both cases, the same difference in percentage cannot be equally detected.

Coming to the major QTL on chromosome 3HL, we found that this locus was responsible for most of the genetic and phenotypic variations in both populations, at coincident positions. SSR- and BOPA-derived CAPS markers confirmed the *Rrs1* locus in both Spanish landrace-derived lines as the main candidate for the outstanding resistance level to scald.

Formerly known as the *Rh-Rh3-Rh4* locus, *Rrs1* was the first scald resistance gene to be reported (Dyck and Schaller 1961; Starling et al. 1971). Graner and Tekauz (1996) identified a dominant resistance gene and located it in an RFLP-based linkage map of chromosome 3HL near the centromere, in the progeny of the DH population Igri × Triton (52 DH lines). In the work by Graner and Tekauz (1996), several RFLP markers co-segregated with the resistance locus *Rrs1*. From one of those RFLPs, the authors developed the co-dominant STS marker cMWG680. The close association of this marker, or the original RFLP (MWG680), has been repeatedly found in literature (Grønnerød et al. 2002; Genger et al. 2003; Patil et al. 2003). The SNP marker 11_0315, 2.4 cM distal to *Rrs1* (Figure 2.3-2), was actually developed from the same EST as cMWG680, so it can be considered to map at the same location for practical purposes. Our position for SNP 11_0315 is consistent with the position of cMWG680 in the four previous studies mentioned. Further investigations of Patil et al. (2003) identified the *Rrs1* resistance locus in the DH population CI11549 × Ingrid. The locus was roughly mapped to the centromeric region of chromosome 3H. The precision of the mapping was impaired by the population size as well as by the presence of a second resistance locus *Rrs4* on the same chromosome. But this second locus was linked to the SSR marker HVM0060 mapping about 22 cM distal to *Rrs1.* Our consensus map with over 800 DH lines (Figure 2.3-2) clearly

places 11_0315 and HVM0060 23 cM apart, about the same distance found in that study, and very similar to the one presented in the dense map of Aghnoum et al. (2010). All these distances are very consistent and, given the high precision of the map of chromosome 3H presented in this work, we can rule out that *Rrs4* segregates in our populations.

The formerly known *Rh-Rh3-Rh4* locus, renamed *Rrs1* in 2002, could be a complex locus for scald resistance. Bjørnstad et al. (2002) identified 11 alleles at the *Rrs1* locus and suggested that there should be more. Recently, Li and Zhou (2011) described two new QTLs at the same location in the TX9425 × Franklin and Yerong × Franklin DH populations. The Franklin allele provided resistance to one population, but susceptibility to the other population. The Yerong allele on 3H showed much better resistance to scald than the Franklin allele, which had not been reported before. These results confirm the presence of an allelic series at this locus, with functional differences at least in some cases. The QTL analyses reported in this work suggest that both SBCC145 and SBCC154 may carry a strong allele of the *Rrs1* locus such as Osiris, Yerong, La Mesita and CIho3515, and more efficient than those found in other cultivars (Table 2.3-1). Actually, Yerong is genetically very closely related to Osiris Langridge et al. 1996), almost as close as to its parent Malebo (Read and Macdonald 1991).

The existence of one or two scald resistance loci in the centromeric region of 3HL has not been settled yet. Classical studies by Dyck and Schaller (1961) and Habgood and Hayes (1971) found a few recombinants in crosses involving *Rh3, Rh4* and other alleles but, according to the latter study, they could not exclude that the recombinants were the result of outcrossing. To settle this issue, new populations of large size involving informative parents should be constructed and studied with a panel of differential scald isolates.

The panel of differential scald isolates derived from the work by Wallwork and Grcic (2011) indicated that the *Rrs1* allele carried by both donors was $Rrs1_{Rh4}$. The evaluation of both SBCC parents revealed that they presented a virulence pattern close to the resistance traditionally described as *Rh4* (Graner and Tekauz 1996), later renamed by Bjørnstad et al. (2002) as $Rrs1_{Rh4}$, to indicate that it belonged to the *Rrs1* locus. This resistance seems typical of accessions originating in North Africa or the Western Mediterranean region—CIho3515 is Spanish, Osiris and Malebo (parent of Yerong) from Algeria and La Mesita is from Egypt— that evolved along one of the possible paths of expansion of barley from the Fertile Crescent towards the West (Baba et al. 2011; Igartua et al. 2013). Therefore, it may have evolved in response to pathotypes prevalent in that region.

Besides a large number of known scald resistance genes, barley researchers still find overall differences between spring and winter barley cultivars regarding the level of scald resistance. Zhan et al. (2008) described that winter barley cultivars apparently have much better resistance to *R. commune* than spring barley cultivars. This could be due to the higher selection pressure on winter barley caused by the longer growing season and the longer period of cold and humid weather in fall and early spring. Therefore, the process of selection for healthier lines is more distinct than in spring barley. This means that in general the resistance level in winter barley tends to be on a higher level. Spanish barleys, though sometimes informally described as spring types, are actually winter types with a reduced vernalization requirement (Casao et al. 2011), and are usually sown in autumn. In this case, SBCC145 is a typical example of that kind of genotype. SBCC154, however, is a true spring barley, the only one of the collection showing any degree of resistance to scald, and is probably one of the most resistant spring barleys described in the literature. It may have arisen as a recombination with resistant winter types prevalent in the region.

To analyse the possible pleiotropic interaction of growth habit and scald, the populations were additionally investigated for a segregating dwarfing gene present on 3HL (as stated by Ponce-Molina et al. (2012) for SBCC145 × Beatrix, and by E. Igartua, unpublished), but no effect of this gene on scald resistance was observed. In this regard, a scald resistance QTL was detected based on field observations and interpreted as a pleiotropic effect of growth habit (Jensen et al. 2002; Looseley et al. 2012). Prostrate plants carrying the dwarfing allele, were more prone to acquire the disease by spread from rain splashes. In our study, done under controlled conditions, plant architecture did not play a role, but this factor should be taken into account when using this germplasm for breeding.

Besides scald, the SBCC lines are an interesting germplasm resource providing ample variability for several agronomic key traits, directly useful for breeding programs. Within the SBCC145 × Beatrix population, lines segregate for spring and winter types, two and six rows, plant height (Beatrix carries the *denso* gene, Ponce-Molina et al. 2012) and for resistance to both scald and powdery mildew (Silvar et al. 2011). This diversity ensures that resistance is selected from amongst a wide variety of plant types.

In summary, we were able to show that the $Rrs1_{Rh4}$ scald resistance locus identified in the lines of the Spanish Barley Core Collection, SBCC145 and SBCC154, is of importance for barley breeding programs. We were able to ascertain the disease reaction with appropriate differentials and to position closely linked markers for the *Rrs1* resistance locus in a complicated chromosomal region with low recombination frequency. The re-

gion encompassing the QTL and markers 11_1476, 11_0010, 11_0823, 11_0205 and 11_0315 was mapped with great confidence, based on a large number of individuals and coincides in loci order with the comprehensive consensus map of Muñoz-Amatriaín et al. (2011). The consensus map developed, combining SSRs, STS and SNPs, presents an improvement of the definition of the *Rrs1* region, including different scald resistance alleles and presents an increase in the precision of the location of *Rrs1* compared with previous reports and their respective markers.

The increased polymorphism granted by the use of two different mapping populations allowed a better resolution of the QTL region than would have been attained by one population alone and pointed to the region flanked by markers 11_0010 and 11_0823 as the most plausible position of the locus. By locating them in the physical map of barley, we were able to define an interval of approximately 8.9 Mb, which contains at least 26 high confidence genes, including two chitinases, usually involved in defense reactions (Collinge et al. 1993). Although the region is close to the centromere, showing low recombination, the future identification of a candidate gene through the development of a large population to search for recombinants in this region, with only around 30 genes, seems feasible.

For marker-based breeding programs, the closely linked BOPA1 SNP markers have been converted to easy accessible CAPS markers. Unfortunately they are still not perfectly diagnostic or functional for the *Rrs1* locus. Nevertheless, the easy to handle CAPS markers developed are all closer to the *Rrs1* gene than formerly known markers. Therefore, we keep looking for further polymorphic markers mapping into the small interval between markers 11_1476 and 11_0205 (which flank the QTL with great certainty in both populations), for which we still found 22 (SBCC145 × Beatrix) and 17 (SBCC154 × Beatrix) recombinant DH lines. A BSTA (bulked segregant transcriptome analysis) approach and the production of marker-selected recombinant F2:3 lines have been started, and the analyses of map-based candidate genes is in process.

2.5. Acknowledgments

This work was funded by the German BMELV and BLE through the project "*Rhynchosporium secalis*-resistance" in barley (project 28-1-41.009-06) and PLANT KBBE II "ExpResBar" AZ 0315702C and by the Spanish Ministry of Science and Innovation (projects GEN2006-28560-E, AGL2007-63625, RFP2012-00015-00-00 and PLANT KBBE "ExpResBar", EUI2009-04075) and co-funded by the European Regional Development Fund. CS held an I3P contract from CSIC. BCM is supported by Fundación ARAID, Zaragoza, Spain. Barley-SSR markers were kindly provided by Andreas Graner and Marion Röder, IPK Gatersleben. Isolates UK7 and AU2 were kindly provided by W. Knogge, Leibnitz Institute for Plant Biochemistry, Halle. The authors would like to thank Alfred Barth and Alexandra Jestadt for the high level of commitment in the laboratory, greenhouse and field and Carlos P. Cantalapiedra for assistance in the work with the physical map of barley. The Grains Research and Development Corporation (GRDC) and SARDI funded the work in Australia.

2.6 Supplementary Material

Table 2.6-1: CAPS markers derived from BOPA1 markers.

Marker	Left primer (5´- 3´)	Right primer (5´- 3´)	Restriction enzyme
11_0010[1]	CGACGAGGAAGATGATGACC	TCAGCACACCACTCCAATGT	*HpaII*
11_0823[1]	CAGTCACCCAAACCTTCGAG	TACATGCGCATCTTGTGATG	*TaqI*
11_0205	GCATTTGGAGGAGTCTGCAT	ACAAACACTTGGCGGCTAAT	*MaeI*
11_0315	GGACAAGTTCAGCACACAGC	ACGAAGTCATGGCAAGCTCT	*AciI*
11_1476	CATCGGCAAAGATAACTCCTG	GCAGAGGAGACAATGGGCTA	*AciI*

[1]Polymorphic only in SBCC154 x Beatrix.

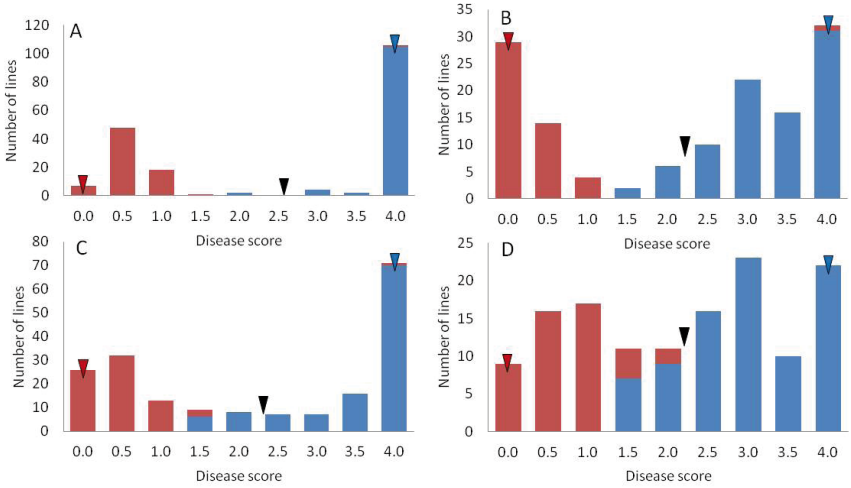

Figure 2.6-1: Response of SBCC145 × Beatrix and SBCC154 × Beatrix DH lines to *R. commune* isolates 271 and LfL07 for the subpopulations genotyped with BOPA1 (n = 190/SBCC145 × Beatrix, n = 168/SBCC154 × Beatrix). The Jackson and Webster (1976) scale extended by half steps was used. In addition occurrence of the marker allele of 11_0205 is shown (red "SBCCxxx", blue "Beatrix" - allele). Black vertical arrows indicate mean disease scores, red vertical arrows the resistant parent SBCC145 or SBCC154 respectively and blue vertical arrows the susceptible parent Beatrix. A) SBCC145 × Beatrix, isolate 271. B) SBCC154 × Beatrix, isolate 271. C) SBCC145 × Beatrix, isolate LfL07. D) SBCC154 × Beatrix, isolate LfL07.

Table 2.6-2: SSR markers assayed in both populations.

Marker name	SBCC145 x Beatrix (cM)	SBCC154 x Beatrix (cM)	Source
HvLTPPB	-	0.0	Ramsay et al. (2000)
GBM1074[1]	-	0.0	Thiel et al. (2003)
Bmag0318b	30.8	-	Ramsay et al. (2000)
GBM1444	32.3	30.3	Varshney et al. (2006)
GBMS0149[2]	34.0	-	Li et al. 2003
Bmag0122	35.1	-	Ramsay et al. (2000)
GBMS0203[2]	35.1	-	Li et al. 2003
Bmag0508	35.8	-	Ramsay et al. (2000)
GBM1413	36.9	-	Varshney et al. (2006)
GBMS0157[2]	38.2	-	Li et al. 2003
GBM1139	38.4	-	Varshney et al. (2006)
Bmac0129	38.7	-	Ramsay et al. (2000)
Bmac0067	-	32.9	Ramsay et al. (2000)
Ebmac0871	39.0	33.4	Ramsay et al. (2000)
GBMS0185[2]	39.0	-	Li et al. 2003
Bmag0136	39.0	33.4	Ramsay et al. (2000)
Bmag0138a	39.0	-	Ramsay et al. (2000)
GBMS0046[2]	39.0	-	Li et al. 2003
GBMS0048[2]	39.0	-	Li et al. 2003
GBMS0189[2]	39.0	-	Li et al. 2003
GBMS0198[2]	39.0	-	Li et al. 2003
Falcon	39.0	33.4	Penner et al. (1996)
HVM0027	39.0	33.4	Liu et al. 1996
GBM1031[1]	39.0	33.4	Thiel et al. (2003)
Bmac0209	-	33.4	Ramsay et al. (2000)
Bmag0006	-	33.4	Ramsay et al. (2000)
GBM1242	41.0	34.3	Varshney et al. (2006)
STSagtc17	48.6	41.6	Grønnerød et al. (2002)
scsnp19175	51.4	-	Rostoks et al. (2005)

GBM1163	54.8	-	Varshney et al. (2006)
GBM1253	56.7	-	Varshney et al. (2006)
HVM0033	56.8	-	Liu et al. 1996
GBMS0022[2]	57.6	-	Li et al. 2003
scind15443	60.7	-	Rostoks et al. (2005)
HVM0060	70.4	-	Liu et al. 1996
GBM1034[1]	71.2	-	Thiel et al. (2003)
Bmag0225	72.6	57.3	Ramsay et al. (2000)
GBM1405	-	62.6	Varshney et al. (2006)
Bmag0013	-	95.3	Ramsay et al. (2000)
GBM1059[1]	-	107.4	Thiel et al. (2003)

[1] PCR primers available upon request to Andreas Graner, IPK Gatersleben

[2] PCR primers available upon request to Marion Roeder IPK Gatersleben

Table 2.6-3: Markers showing segregation distortion in the region of chromosome 3H encompassing the scald resistance QTL. Closest markers in the region of *Rrs1* are marked with a box in each population.

Population SBCC145xBeatrix

			Absolute frequencies		**Relative frequencies**	
Chromosome	**Marker**	**Position**	**SBCC145**	**Beatrix**	**SBCC145**	**Beatrix**
3H	11_0145	21.184	92	92	0.50	0.50
3H	11_0603	36.674	80	104	0.43	0.57
3H	11_0436	40.552	77	107	0.42	0.58
3H	11_0570	45.234	73	111	0.40	0.60
3H	11_0037	46.927	74	108	0.41	0.59
3H	11_0122	53.361	67	117	0.36	0.64
3H	11_0985	54.971	66	118	0.36	0.64
3H	11_0163	55.541	65	119	0.35	0.65
3H	11_1044	55.541	65	119	0.35	0.65
3H	11_1258	57.560	62	122	0.34	0.66
3H	11_1156	58.647	60	124	0.33	0.67
3H	11_0652	59.709	60	124	0.33	0.67

3H	11_1091	60.237	59	124	0.32	0.68
3H	11_0827	63.618	61	123	0.33	0.67
3H	11_0407	64.058	62	121	0.34	0.66
3H	11_1096	64.061	62	122	0.34	0.66
3H	11_1105	64.061	62	122	0.34	0.66
3H	11_0461	65.530	62	120	0.34	0.66
3H	11_1153	65.549	62	121	0.34	0.66
3H	11_1443	65.549	62	121	0.34	0.66
3H	11_1341	65.549	62	121	0.34	0.66
3H	11_1211	65.549	62	121	0.34	0.66
3H	11_1338	65.551	63	120	0.34	0.66
3H	11_1518	65.551	63	121	0.34	0.66
3H	11_1063	65.551	63	121	0.34	0.66
3H	11_0187	65.551	63	121	0.34	0.66
3H	11_1356	65.551	63	121	0.34	0.66
3H	11_1533	65.551	63	121	0.34	0.66
3H	11_1154	65.551	63	121	0.34	0.66
3H	11_1421	65.551	63	121	0.34	0.66
3H	11_0920	65.551	63	121	0.34	0.66
3H	11_0679	65.551	63	121	0.34	0.66
3H	11_0546	65.551	63	121	0.34	0.66
3H	11_0302	65.551	63	121	0.34	0.66
3H	11_0365	65.551	63	121	0.34	0.66
3H	11_0417	65.551	63	121	0.34	0.66
3H	11_0250	65.551	63	121	0.34	0.66
3H	11_0774	65.551	63	121	0.34	0.66
3H	11_0779	65.551	63	121	0.34	0.66
3H	11_0504	65.551	63	121	0.34	0.66
3H	11_0022	65.551	63	121	0.34	0.66
3H	11_1519	65.551	63	121	0.34	0.66
3H	11_0013	65.551	63	121	0.34	0.66
3H	11_0820	65.551	63	121	0.34	0.66
3H	11_0002	65.551	63	121	0.34	0.66

3H	11_0989	65.551	63	121	0.34	0.66
3H	11_0411	65.551	63	121	0.34	0.66
3H	11_1296	65.551	63	121	0.34	0.66
3H	11_1355	65.551	63	121	0.34	0.66
3H	11_1476	67.172	66	118	0.36	0.64
3H	11_0205	72.078	73	109	0.40	0.60
3H	11_0315	72.594	74	110	0.40	0.60
3H	11_1378	73.163	73	111	0.40	0.60
3H	11_1013	74.876	76	108	0.41	0.59
3H	11_1470	76.591	77	107	0.42	0.58
3H	11_1472	77.168	78	106	0.42	0.58
3H	11_1197	77.168	78	106	0.42	0.58
3H	11_0373	77.168	78	106	0.42	0.58
3H	11_0963	79.972	77	107	0.42	0.58
3H	11_1401	80.993	77	107	0.42	0.58
3H	11_0493	82.176	79	105	0.43	0.57
3H	11_0308	82.176	79	105	0.43	0.57
3H	11_1240	86.896	85	99	0.46	0.54

Population SBCC154xBeatrix

			Absolute frequencies		Relative frequencies	
Chromosome	**Marker**	**Position**	**SBCC154**	**Beatrix**	**SBCC154**	**Beatrix**
3H	11_0557	15.673	85	83	0.51	0.49
3H	11_0519	35.112	70	98	0.42	0.58
3H	11_0772	36.459	70	98	0.42	0.58
3H	11_0917	36.459	70	98	0.42	0.58
3H	11_0436	37.810	68	100	0.40	0.60
3H	11_0163	52.520	59	109	0.35	0.65
3H	11_1062	55.467	58	110	0.35	0.65
3H	11_0613	55.467	58	110	0.35	0.65
3H	11_1156	56.044	57	110	0.34	0.66
3H	11_1258	56.075	57	111	0.34	0.66
3H	11_0633	56.075	57	111	0.34	0.66

3H	11_0652	58.967	56	112	0.33	0.67
3H	11_1023	58.967	56	112	0.33	0.67
3H	11_0407	60.166	56	112	0.33	0.67
3H	11_1096	60.763	57	111	0.34	0.66
3H	11_0365	62.473	54	113	0.32	0.68
3H	11_1153	62.473	52	114	0.31	0.69
3H	11_1410	62.480	54	113	0.32	0.68
3H	11_1356	62.483	54	114	0.32	0.68
3H	11_0504	62.483	54	114	0.32	0.68
3H	11_0411	62.483	54	114	0.32	0.68
3H	11_1211	62.483	54	114	0.32	0.68
3H	11_1518	62.483	54	114	0.32	0.68
3H	11_1341	62.483	54	114	0.32	0.68
3H	11_0187	62.483	54	114	0.32	0.68
3H	11_0417	62.483	54	114	0.32	0.68
3H	11_0779	62.483	54	114	0.32	0.68
3H	11_0013	62.483	54	114	0.32	0.68
3H	11_1338	62.483	54	114	0.32	0.68
3H	11_0920	62.483	54	114	0.32	0.68
3H	11_0250	62.483	54	114	0.32	0.68
3H	11_0002	62.483	54	114	0.32	0.68
3H	11_0302	62.483	54	114	0.32	0.68
3H	11_0820	62.483	54	114	0.32	0.68
3H	11_0546	62.483	54	114	0.32	0.68
3H	11_1047	62.483	54	114	0.32	0.68
3H	11_1443	62.483	54	114	0.32	0.68
3H	11_1063	62.483	54	114	0.32	0.68
3H	11_1533	62.483	54	114	0.32	0.68
3H	11_0774	62.483	54	114	0.32	0.68
3H	11_0461	62.483	54	114	0.32	0.68
3H	11_0262	62.483	54	114	0.32	0.68
3H	11_1421	62.483	54	114	0.32	0.68
3H	11_1355	62.483	54	114	0.32	0.68
3H	11_1296	62.483	54	114	0.32	0.68

3H	11_0989	62.483	54	114	0.32	0.68
3H	11_0679	62.483	54	114	0.32	0.68
3H	11_0022	63.642	54	114	0.32	0.68
3H	11_1469	63.642	54	114	0.32	0.68
3H	11_0263	63.642	54	114	0.32	0.68
3H	11_1154	63.642	54	114	0.32	0.68
3H	11_1476	63.642	54	114	0.32	0.68
3H	11_0243	64.227	55	113	0.33	0.67
3H	11_1282	64.752	56	112	0.33	0.67
3H	11_0724	64.752	56	112	0.33	0.67
3H	11_0010	68.498	60	108	0.36	0.64
3H	11_0823	71.885	62	106	0.37	0.63
3H	11_0205	73.069	62	106	0.37	0.63
3H	11_0401	73.069	62	106	0.37	0.63
3H	11_0315	75.161	64	103	0.38	0.62
3H	11_1197	76.671	64	104	0.38	0.62
3H	11_0373	76.671	64	104	0.38	0.62
3H	11_0321	79.226	65	103	0.39	0.61
3H	11_0018	79.276	65	102	0.39	0.61
3H	11_0216	79.354	65	101	0.39	0.61
3H	11_0963	79.548	64	104	0.38	0.62
3H	11_0493	81.387	64	104	0.38	0.62
3H	11_0691	91.632	66	102	0.39	0.61
3H	11_0118	95.233	65	103	0.39	0.61
3H	11_1183	95.233	65	103	0.39	0.61
3H	11_0009	109.455	76	92	0.45	0.55

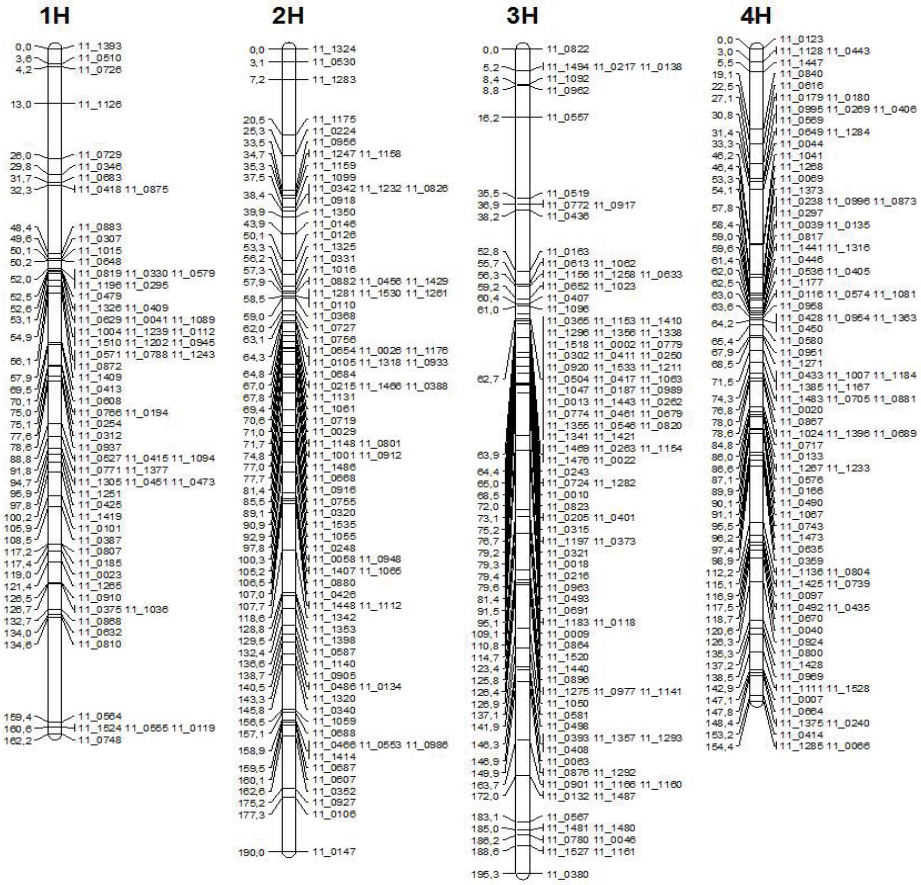

Figure 2.6-2: Genetic map of the population SBCC154 x Beatrix, genotyped with BOPA1.

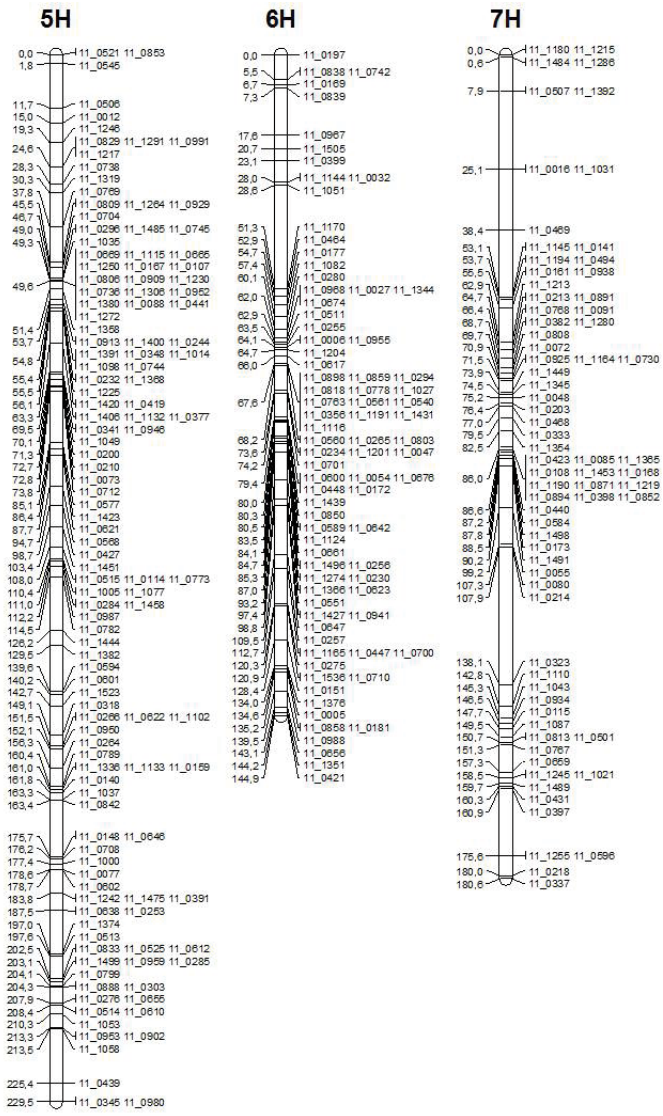

Figure 2.6-02 continued.

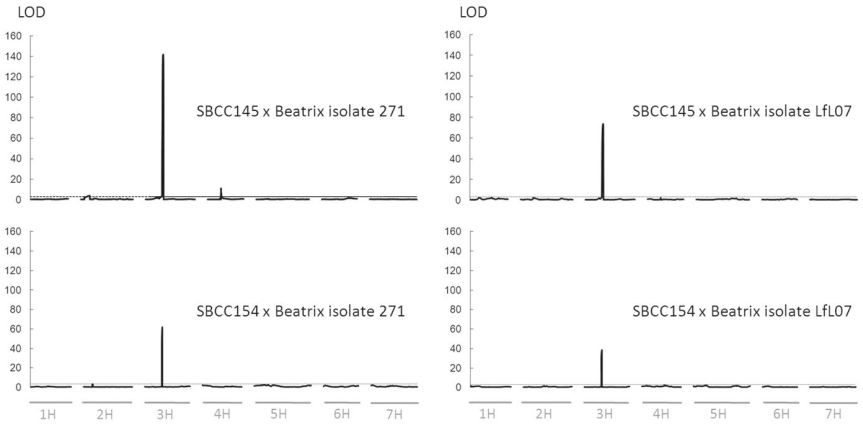

Figure 2.6-3: MQM LOD scans for resistance scores to *R. commune* isolates 271 and LfL07 in 190 DH lines of SBCC145 × Beatrix and 168 DH lines of SBCC154 × Beatrix. The horizontal dotted lines and the numbers above them indicate the significance threshold for QTL detection based on an experiment-wise error rate of less than 5 %, estimated with 1,000 permutations.

3 Chapter B: Genetic mapping and phenotypic assessment of the genes conferring resistance to leaf scald in barley accession CIho3515

3.1 Introduction

Many studies have been conducted to identify genes conveying scald resistance and locate them on the genome. More recent studies used various sources of resistance such as wild barley (Abbott et al. 1995, Garvin et al. 2000, Genger et al. 2005), *Hordeum bulbosum* (Pickering et al. 2006) or landrace collections (Silvar et al. 2010, Hofmann et al. 2013). But for several decades starting in the 1950s the focus was laid on a rather limited set of barley cultivars, the core set of which has been described by Goodwin et al. (1990).

The barley accession CIho3515 has been part of this informal, but nevertheless well established set of genotypes and has been used for scald research ever since the late 1950s. Back then a number of studies attempted to identify the resistance loci underlying observed resistance using differential isolates and a limited number of phenotypic markers (Schein 1958, Dyck and Schaller 1961, Wells and Skoropad 1963, Habgood and Hayes 1971). The consistent message from these works is, that one of the loci present in CIho3515 is an allele of the *Rh-Rh3-Rh4*-locus, today described as *Rrs1* (Bjørnstad et al. 2002) located in the centromeric region of chromosome 3H.

Habgood and Hayes (1971) and Starling et al. (1971) reported the presence of a second resistance gene. While Starling et al. (1971) only excluded the *Rh2* locus (now *Rrs2*, cf. Bjørnstad et al. 2002), Habgood and Hayes (1971) proposed it to be a new resistance gene, named *Rh10*. They also reported the same second locus to be present in a number of varieties, among them Osiris and Atlas46. Today the second locus beside *Rrs1* in these two cultivars is confirmed as *Rrs2* (Hanemann et al. 2009). Consequently the assumption that the second locus of Osiris, Atlas 46 and CIho3515 were identical contradicts the findings of Starling et al. (1971).

As indicated in chapter 2.1, Wallwork and Grcic (2011) distinctly differentiate between *Rrs1* which they consider equal to *Rh/Rh3*, and *Rh4/Rh10*, closely located but not identical to *Rrs1*. According to their phenotypic observation and interpretation of the available literature the resistance locus on chromosome 3H of Osiris and CIho3515 is the *Rh4/Rh10* locus while Atlas46 carries the *Rrs1* locus. The findings of Starling et al. (1971) that CIho3515 carries a second locus different from those of Osiris and Atlas46 were also confirmed by Wallwork and Grcic (2011) at the South Australian Research and Development Institute (SARDI).

While the exact genetic nature of the CIho3515 resistance remains unclear, its outstanding performance has been reported in many geographies in studies mainly concerned with pathogenicity and race variation of *Rhynchosporium commune*: It displayed resistance to all or a considerable number of isolates tested e.g. in Canada (Tekauz 1991), Norway (Salamati and Tronsmo 1997), South Africa (Robbertse et al. 2000) Australia (Ali et al. 1976, Wallwork and Grcic 2011) and Japan (Fukuyama et al. 1998).

This outstanding performance of CIho3515 in various resistance assessments around the globe makes the underlying resistance loci highly valuable for resistance breeding programs in barley. This value is further highlighted by the potential difference of both loci from the by now well described *Rrs1* and *Rrs2* loci (Hofmann et al. 2013 and Hanemann et al. 2009, respectively). The objective of this work was therefore to locate the loci underlying the scald resistance of CIho3515 in the barley genome, and if possible make them accessible for marker-assisted breeding programs.

3.2 Material & Methods

3.2.1 Plant Material and fungal isolates

CIho3515 is a six-rowed spring barley accession from Spain, stored in the USDA World Barley Collection. It was selected for this project because of its outstanding resistance to scald. Susceptible parents for generating crosses (F1) and DH populations thereof were Steffi (Ackermann Saatzucht, Irlbach, Germany) and Alexis (Saatzucht Josef Breun, Herzogenaurach, Germany), both two-rowed spring barley cultivars with good malting quality . Both cultivars are highly susceptible to scald. The cultivar Hendrix, a two-rowed spring barley with good malting quality (KWS LOCHOW, Bergen, Germany).was used as the susceptible check in the field assessment of the population DH33349 (CIho3515 x Alexis).

The DH population derived from a cross between CIho3515 and Steffi (DH761) was already available at the Bavarian State Research Center for Agriculture (LfL). This population, consisting of 75 DH lines, was used to identify the resistance loci. A new DH population was produced by anther culture in 2008/2009 from a cross between CIho3515 and Alexis (DH33349). This population consists of 245 DH lines and was used for validation of the identified loci and a high resolution mapping attempt with focus on the respective genomes.

Five single spore isolates of *R. commune* from the collection of the LfL were used for assessment of scald resistance in the DH populations. Isolates 271 and Sachs147-1 originally came from the collection of the former Federal Institute for Resistance Genetics in Grünbach, Bavaria. Whereas it is known that 271 was collected in Strassmoos, Bavaria, the exact collection site of Sachs147-1 has not been recorded. LfL07 was collected in Freising, Bavaria, in 2007. Isolates Rhy017 and Rhy174 were kindly provided by the James Hutton Institute (the former Scottish Crop Research Institute). Isolate 271 was used for resistance assessment of DH761, while population DH33349 was tested with isolates Sachs147-1, LfL07, Rhy017 and Rhy174. Isolate Sachs147-1 was selected to replace isolate 271 due to some low sporulation being observed in the latter isolate at the time of the resistance assessment. Both isolates have consistently returned similar resistance reactions across many barley genotypes in experiments conducted at the LfL over the last two decades (unpublished data).

3.2.2 Resistance testing

Preparation of the spore suspensions and resistance assessments were carried out as described in chapter 2.2 with one exception: for DH33349 the greenhouse tests were conducted with two plants per DH line and replicate only.

Population DH33349 was also tested once in the field with two randomized replications. DH lines were grown in double lines, two lines always flanking a susceptible standard (Figure 3.2-1). Infection was achieved by chaffing infected barley plants and spreading them amongst the test plots during tillering (BBCH 25). Sufficient humidity was achieved by an irrigation system. Plants were rated approximately 6-8 weeks after infection estimating the infected leaf area on the recommended EPPO scale of 1 (resistant) to 9 (susceptible).

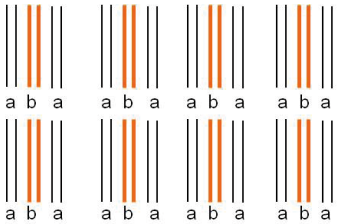

Figure 3.2-1: Experimental set-up of the field test for DH33349. Lines in black (a) represent tested DH lines, lines in bold orange (b) represent the susceptible standard Hendrix.

3.2.3 Statistical analysis

The statistical analysis of the DH761 greenhouse test results was conducted using the mean values of all four plants. In the case of DH33349 graphs and population means were generated for each replicate and isolate using the mean value of both plants per replicate. After determining the correlation between both replicates per isolate, the mean value over both replicates per isolate was used for further analysis.

The correlation between replicates was determined by calculating the coefficient of determination (R^2) values for each isolate to check the reproducibility between replicates for each isolate.

The X^2 test was used to test different hypotheses with respect to the genetics underlying the observed resistance. The hypotheses were set to test the possibilities of one or two gene loci providing full or partial resistance and are described in table 3.2-1. Significance levels tested were alpha = 0.1, 0.05 and 0.01.

Pearson's contingency coefficient (C) was employed to determine the correlation of genetic markers with the observed resistance/susceptibility. C values were divided by C_{max} values, the highest possible C value achievable in the respective experiment. The result is C_{korr}, which is comparable between experiments and lies on a scale between 0 and 1.

Table 3.2-1 Hypotheses for the inheritance of resistance/susceptibility observed in the segregating populations.

Hypo-thesis	Description	Locus 1*	Locus 2*	Expected segregation
H1	2 resistance loci in the population both conveying full resistance and both segregating 1:1	full	full	3 : 1
H2	2 resistance loci in the population one conveying full resistance, one partial resistance and both segregating 1:1	full	partial	2 : 1 : 1
H3	2 resistance loci in the population both conveying partial resistance and both segregating 1:1	partial	partial	1 : 2 : 1
H4	1 resistance locus in the population conveying full resistance, segregating 1:1	full	none	1 : 1
H5	1 resistance locus in the population conveying partial resistance, segregating 1:1	none	partial	1 : 1

*level of resistance conveyed by the respective locus

3.2.4 Genetic analysis

DNA extraction and sources for marker selection have been described in chapter 2.2. Sequences for U35 and H35 SNP markers were kindly provided by M. Loosely (James Hutton Institute, Scotland, sequences on request). SNP markers were converted to CAPS markers using the program SNP2CAPS (Thiel et al. 2004).

An initial screen for known resistance loci in population DH761 was conducted using microsatellite marker HVM0027 for *Rrs1* (Patil et al. 2003) and STS markers ABG320 for *Rrs2* (Schweizer et al., unpublished) and GemS13 for $Rrs15_{CIho8288}$ (Schweizer et al. 2004, Wagner et al. 2008).

An AFLP pool screening approach was used to identify the second resistance locus of CIho3515. DH lines not carrying the *Rrs1* resistance gene were selected with microsatellite marker HVM0027. Ten and eight DH lines with the lowest and highest disease scores respectively were combined into a resistant and a susceptible pool.

AFLP analysis of both pools and the parent accessions was carried out as described in Vos et al. (1995) and Hartl et al. (1999). The S00/M02 primer subset from the PstI/MseI enzyme system consisting of 256 combinations was used, S00 equaling P03 according to the KeyGene nomenclature (http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html). Primer combinations which amplified cosegregating fragments in the pools and respective parents were used again for amplification in the individual lines.

Cosegregating AFLP fragments were cut from the gel and cloned with the QuiaGen Cloning Kit according to manufacturer's instructions. Cloned fragments were sent for sequencing to MWG Eurofins, Ebersberg, Germany. For sequence analysis the software CLC sequence viewer (from version 6.0, CLC Inc, Aarhus, Denmark, www.clcbio.com) was used, primers were designed with the software Primer3 (from version 1.1.0, Untergasser et al. 2012, Koressaar & Remm 2007).

Genetic maps were created as described in chapter 2.2. For the QTL analyses the program PlabQTL (Utz & Melchinger 1996, https://www.uni-hohenheim.de/plantbreeding/software/) was employed. Empirical threshold values for LOD scores were produced by a permutation test with 1,000 replications (Churchill and Doerge 1994). Linkage maps and QTL charts were produced with the program MapChart (version 2.2, Voorrips 2002).

3.3 Results

3.3.1 DH761 – CIho3515 x Steffi

3.3.1.1 Phenotypic assessment

Population DH761 (CIho3515 x Steffi) was assessed for resistance in the greenhouse as described using scald isolate 271. 70 of the 75 DH lines germinated and developed seedlings suitable for disease rating. The distribution of the DH lines to the disease scores is shown in figure 3.3-1.

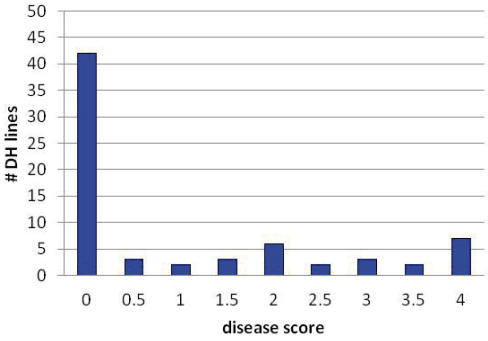

Figure 3.3-1: Response of DH761 lines to *R. commune* isolate 271.

The overall mean of population DH761 is 1.01, with 0 being the lowest and 4 the highest score rated. Average scores for the parents derived from eight plants each are 0.00 for CIho3515 and 3.96 for Steffi. The skewed distribution suggests that the scald reaction is possibly not based on a single locus segregating 1:1 for the resistant and susceptible alleles. Subsequently, five different hypotheses concerning the underlying genetics as described in table 3.2-1 were tested using the X^2 test of goodness of fit. Hypotheses H1 and H2 are accepted at different significance levels but not H3-H5 (table 3.3-1).

Table 3.3-1: Results of X^2 tests for the genetic segregation of scald reaction in population DH761.

	Hypotheses				
	H1	**H2**	**H3**	**H4**	**H5**
X^2 value	0.47619	8.257143	67.91429	12.85714	30.22857
Significance	*	***	-	-	-

*, **, *** hypothesis is accepted at alpha = 0.1, 0.05 or 0.01, respectively; - hypothesis is rejected on all significance levels.

3.3.1.2 Initial marker screening

In order to identify any known resistance gene potentially being present in CIho3515 the population was screened with three markers associated with the major scald resistance genes known in cultivated barley today, *Rrs1*, *Rrs2*, and $Rrs15_{CIho8288}$. As described in chapter 3.2.4, markers for these genetic loci were selected as follows: SSR marker HVM0027 for *Rrs1* and STS markers ABG320 for *Rrs2* and GemS13 for $Rrs15_{CIho8288}$.

Correlation between those markers and the observed resistance was determined using Pearson's contingency coefficient. C_{max} was determined as 0.71, calculated C values and C values corrected by C_{max} are shown in the following table 3.3-2.

Table 3.3-2: Contingency coefficients for markers HVM0027, ABG320 and GemS13. C_{corr} values are C values corrected by C_{max}.

	HVM0027	**ABG320**	**GemS13**
C	0.61	0.40	0.34
C_{corr}	0.86	0.57	0.48

HVM0027 displays a considerable correlation with the observed resistance. This potential linkage with the resistance observed in DH761 is supported by the results from a marker assisted selection attempt carried out with HVM0027. All DH lines containing the allele of HVM0027 coming from the resistant parent CIho3515 are resistant as well with disease scores of 1.5 or lower (see figure 3.3-2). The mean infection score for this subpopulation is 0.06 as compared to 1.01 for the whole DH population.

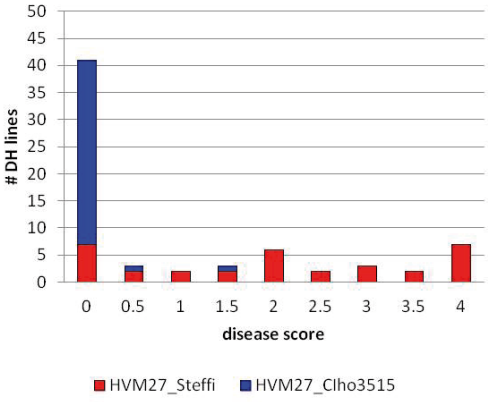

Figure 3.3-2: Response of DH761 lines to *R. commune* isolate 271. In addition the distribution of STS marker HVM0027 in the population is displayed. Blue bars indicate the number of lines carrying the allele from the resistant parent CIho3515; red bars represent DH lines carrying the allele from the susceptible parent Steffi.

3.3.1.3 AFLP pool screening

As described in chapter 3.2.4, an AFLP poolscreening approach was employed to locate the second resistance locus in the genome. Pools were assembled from the subpopulation carrying the HVM0027 allele coming from susceptible parent Steffi (figure 3.3-2, shown in red). The most promising primer combination used in the poolscreening approach was S20M48. One of the amplified fragments is polymorphic between parents CIho3515 and Steffi, and the resistant and susceptible pools cosegregate with the respective parents (figure 3.3-3). Amplification of primer combination S20M48 in the individual lines from both pools confirmed this result (Figure 3.3-4).

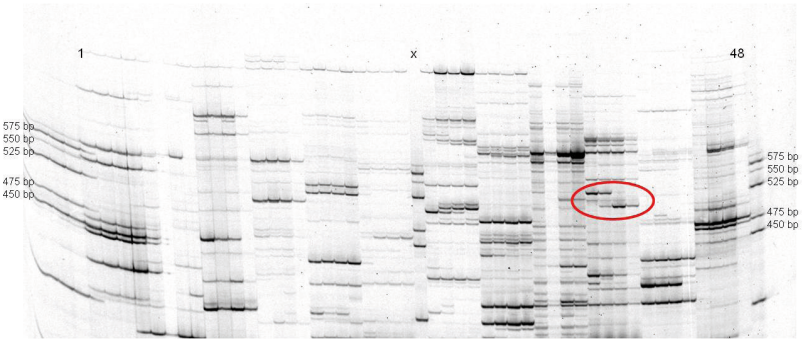

Figure 3.3-3: PCR products from parents and DH line pools generated with 12 different AFLP primer combinations. Primer combination S20M48 is indicated by a red circle. “1” indicates the first line of the assay, “48” the last lane. Always four lanes belong to the same primer combination. Lanes per primer combination from left to right: CIho3515, resistant pool, susceptible pool, Steffi. Left and right of the assay and at position “x” DNA standards were added.

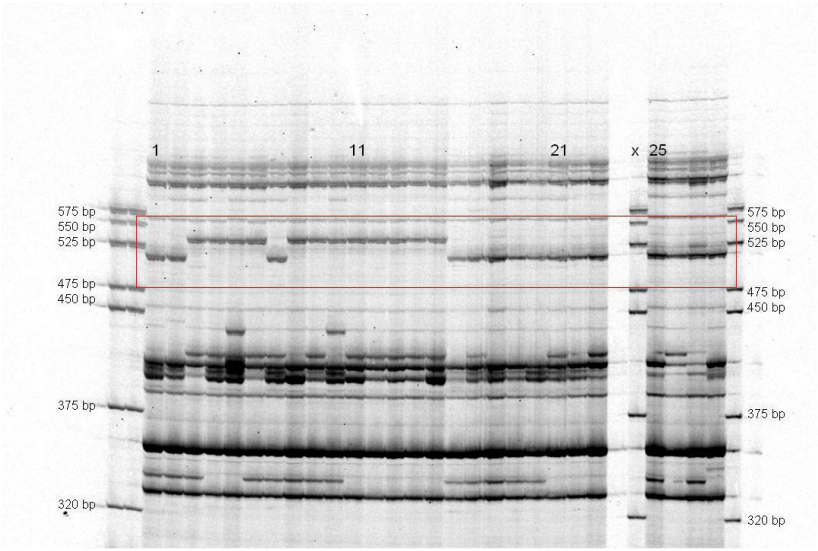

Figure 3.3-4: PCR products for primer combination S20M48 from the single lines that were used to assemble the pools. Lanes 1-10: resistant pool. Lanes 11-15: CIho3515, lanes 16-20: Steffi, lanes 21-28 (excluding “x”): susceptible pool. Left and right of the assay and at position “x” DNA standards were added. DH lines in lanes 1, 2 and 7 where later on excluded from the population, as too many PCR fragments were amplified during genotyping, which did not belong to either of the parents.

The fragments were cloned and sequenced as described in chapter 3.2.4. Alignment of the sequences reveals two indels of 30 and 5 bp (figure 3.3-5). Primers flanking the larger indel were designed (table 3.3-3) to create a STS marker, denominated as 2048_2. This marker produces **213** and **184** bp PCR fragments for resistant and susceptible genotypes respectively (figure 3.3-6).

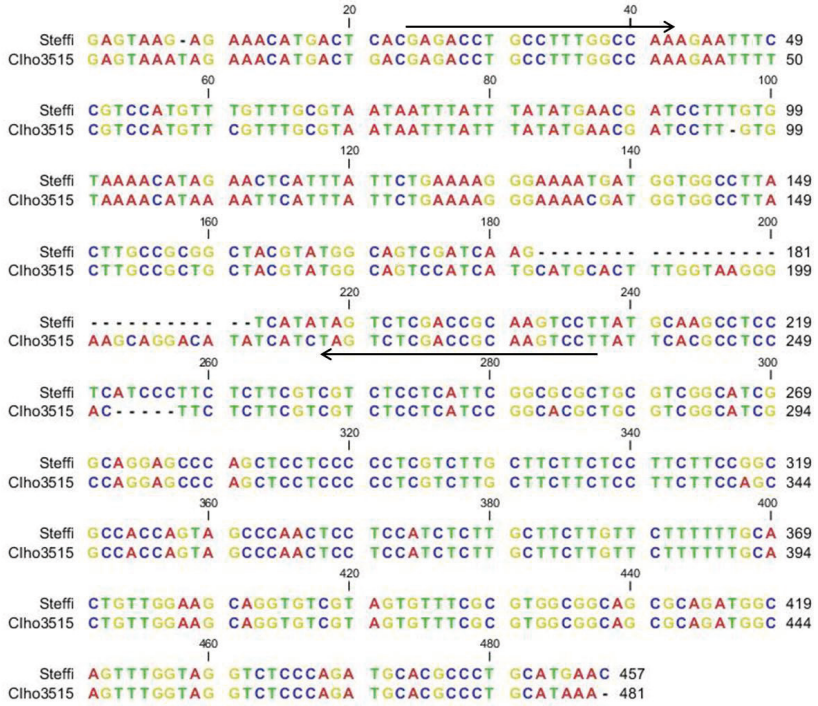

Figure 3.3-5: Sequence of the cloned AFLP fragments amplified with primer combination S20M48 in Steffi and CIho3515. Black arrows indicate primer binding sites for STS marker 2048_2 (see also table 3.3-3).

Table 3.3-3: Primer sequences for STS marker 2048_2.

Primer	Sequence	Annealing temperature
2048_2_F	GAGACCTGCCTTTGGCCAA	60°C
2048_2_R	AGGACTTGCGGTCGAGACTA	60°C

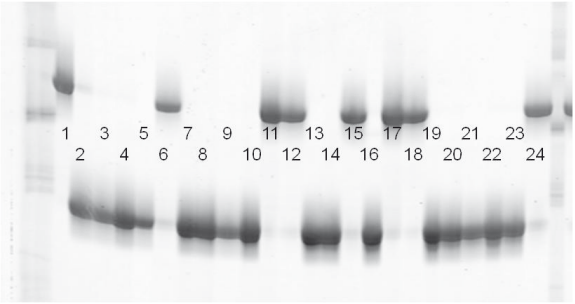

Figure 3.3-6: PCR fragment pattern generated by STS marker 2048_2 on 22 DH lines of DH 761 (1-22) and their parents, Steffi (23) and CIho3515 (24).

Correlation of this marker with the observed resistance was determined as well using Pearson's contingency coefficient. Values for 2048_2 are C = 0.50 and C_{korr} = 0.71, reflecting a distinct correlation of the marker with the resistance observed in DH761. Marker 2048_2 was also used for marker assisted selection (MAS) in DH761 (figure 3.3-7). The resulting subpopulation with the resistance-associated allele has a mean disease score of 0.59 as compared to 1.01 for the whole population. The subpopulation contains fully resistant DH lines as well as DH lines showing only partial resistance with disease scores of 2 to 2.5, indicating that the second locus present in CIho3515 might not be fully effective against *R. commune*.

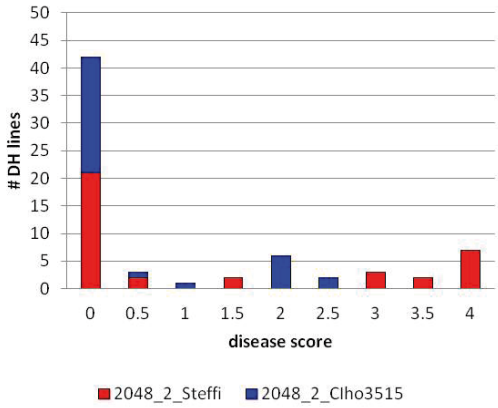

Figure 3.3-7: Response of DH761 lines to *R. commune* isolate 271. In addition the distribution of STS marker 2048_2 in the population is displayed. Blue bars indicate the number of lines carrying the allele from the resistant parent CIho3515; red bars represent DH lines carrying the allele from the susceptible parent Steffi.

3.3.1.4 Genetic mapping

To locate the second resistance locus in the genome, the population DH761 was genotyped with 98 genetic markers distributed over the whole genome, including STS marker 2048_2. A total of 82 markers were mapped into 13 linkage groups covering 369.43 cM (figure 3.3-8). The linkage groups were assigned to chromosomes by comparison with consensus maps from Ramsay et al. 2000, Varshney et al. 2007, Marcel et al. 2007 and using the information available in the GrainGenes data base (http://wheat.pw.usda.gov/GG2/index.shtml). STS marker 2048_2 maps to the distal part of the short arm of a linkage group corresponding to chromosome 6H (figure 3.3-8), although cosegregating SSR marker GBM1067 was assigned to chromosome 4H in the past by Stein et al. (2007) and Varshney et al. (2007).

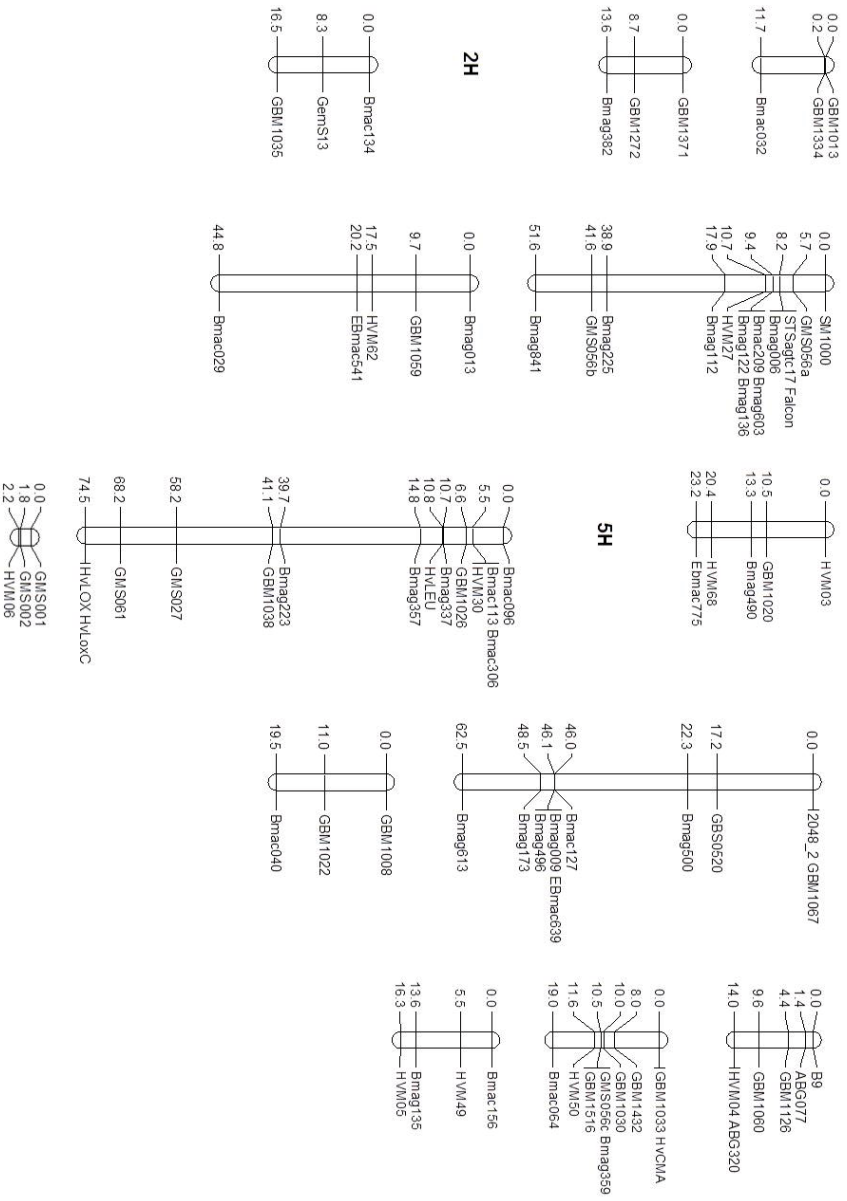

Figure 3.3-8: Genetic map of barley population DH761 (CIho3515 x Steffi).

3.3.1.5 QTL analysis of scald reaction

For validation purposes a QTL analysis was conducted. The critical LOD score for this experiment was determined as 2.40. The first analysis was carried out without any co-factors. It returned only one QTL on chromosome 3H with a LOD score of 10.9 and explaining 51.2% of the phenotypic variation (R^2 %). The analysis was repeated using STS marker 2048_2 as cofactor for the potential resistance locus on chromosome 6HS, and STSagtc17 for the putative *Rrs1* allele on chromosome 3H. The second marker was selected based on the results from Hofmann et al. (2013) (see chapter 2).

Two QTL were detected in this second analysis on chromosomes 3H and 6H. LOD scores, R^2 % and additive effects are presented in table 3.3-4. Locations of the QTL on the respective linkage group or chromosome are indicated in figure 3.3-9.

Table 3.3-4: Quantitative trait loci (QTL) detected in population DH761 (CIho3515 x Steffi) after inoculation with *R. commune* isolate 271 and using cofactors STSagtc17 and 2048_2. The location of the QTL in the barley genome is presented in figure 3.3-9.

QTL	LOD score	R^2 %	Additive effect
271_3H	12.23	55.3	-1.045
271_6H	2.68	16.6	-0.471

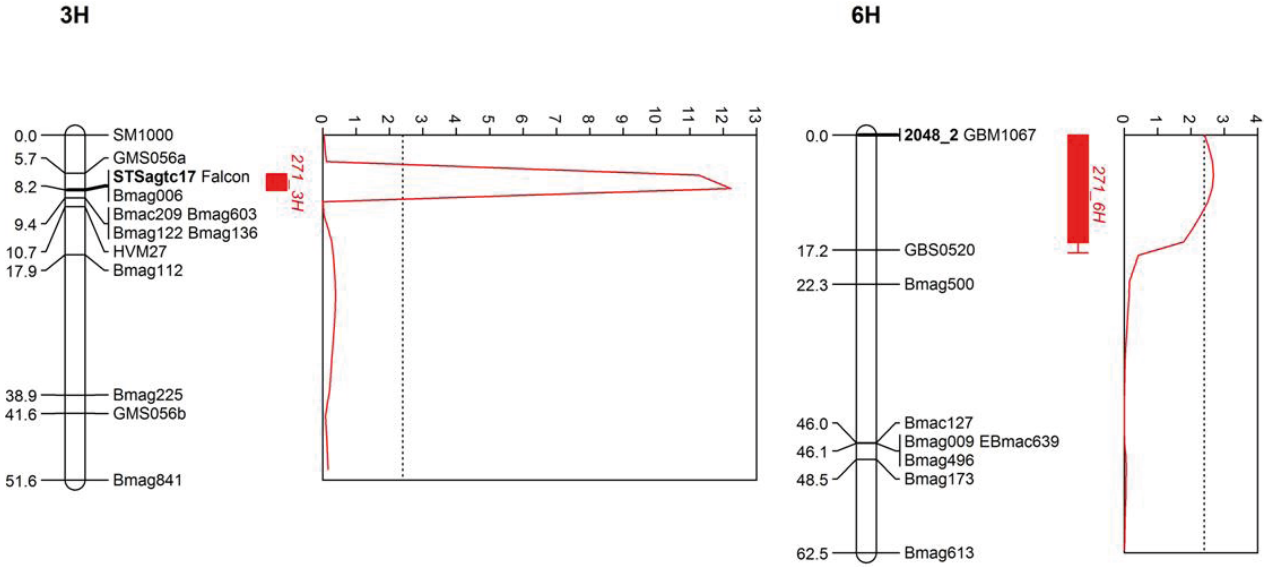

Figure 3.3-9: Location of the detected QTL for *R. commune* resistance on the respective linkage groups (chromosomes) of barley population DH761.

3.3.2 DH33349 – Clho3515 x Alexis

3.3.2.1 Phenotypic assessment

Population DH33349 was assessed for scald resistance in the greenhouse with four different isolates of *R. commune*: Sachs147-1, LfL07, Rhy174 and Rhy017. The number of DH lines rated in each experiment, the respective population means and the disease scores for the parents are summarized in table 3.3-5. The parent scores are averages from 12 individual plants per experiment.

Table 3.3-5: Number of DH lines, mean disease scores and disease scores for each isolate of the parents are presented for replicates 1 and 2.

Isolate	**n=***	**Population mean**	**Clho3515**	**Alexis**
	R1 / R2**	R1 / R2*	R1 / R2*	R1 / R2*
Sachs147-1	232 / 232	0.79 / 0.85	0 / 0	4 / 4
LfL07	233 / 224	1.58 / 1.58	0 / 0	4 / 4
Rhy174	229 / 234	2.61 / 2.59	0.37 / 0.52	3.98 / 3.98
Rhy017	237 / 234	1.36 / 1.41	0 / 0	4 / 4

*deviations from total population size are caused by low germination rate of some DH lines

**R1 = replicate1, R2 = replicate 2

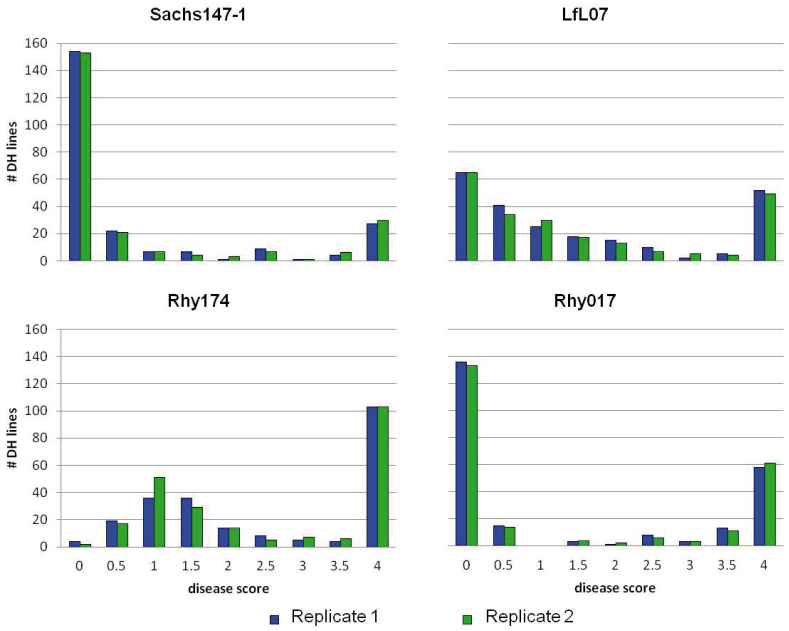

Figure 3.3-10 Response of DH33349 lines to *R. commune* isolates Sachs147-1 (top left), LfL07 (top right), Rhy174 (bottom left) and Rhy017 (bottom right). Results for the first replicate are displayed in blue, results for the second replicate are displayed in green.

The diagrams in figure 3.3-10 display the distribution of the DH lines to the disease scores. When comparing replicate 1 with replicate 2 for each isolate, a high similarity between distributions can be observed. Distribution patterns however vary considerably between isolates.

Scatter plots were generated for all four isolates by plotting the disease score of replicate 1 against the score from replicate 2 for each DH line (Figure 3.3-11). The resulting diagram indicated a high correlation between experiments. R^2 values were determined to confirm this. The resulting values ranged between 0.96 and 0.99 and led to the decision to use the mean values over both replicates for all subsequent analyses.

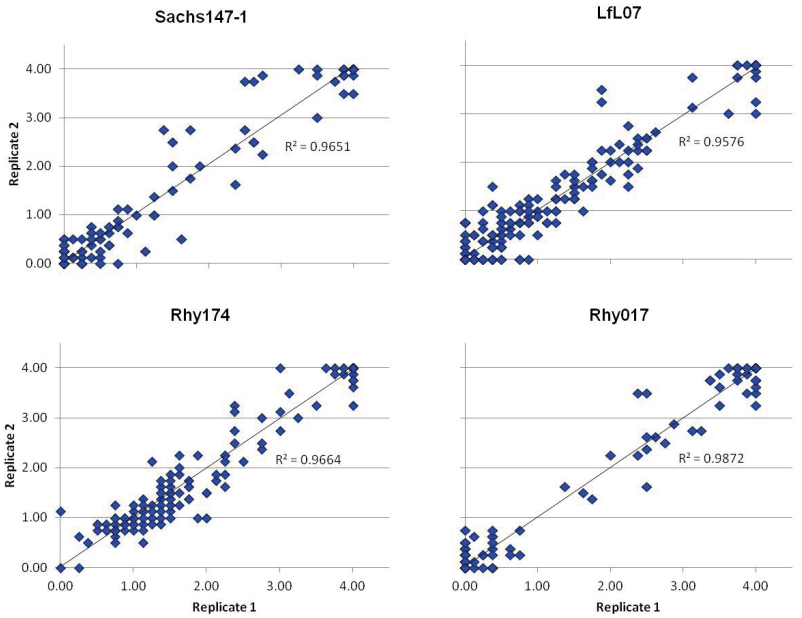

Figure 3.3-11 Disease scores for each DH line of replicate 1 are plotted against the disease scores of replicate 2 for the respective DH line. Resulting scatter plots display the correlation of both replicates for experiments with isolates Sachs147-1 (top left), LfL07 (top right), Rhy174 (bottom left) and Rhy017 (bottom right) respectively. R^2 was determined, revealing correlations between the replicates of 0.97, 0.96, 0.97 and 0.99, respectively.

Figure 3.3-12 displays the distribution of the DH lines to the disease scores for each isolate using the mean values over both replicates. Overall numbers of lines assessed, population means and parent disease scores over both replicates for each isolate are presented in table 3.3-6.

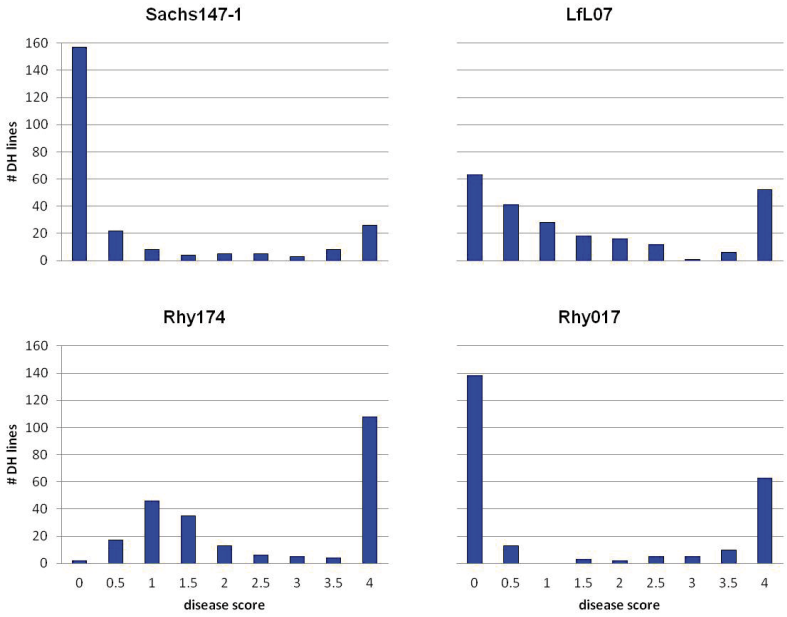

Figure 3.3-12: Response of DH33349 lines to *R. commune* isolates Sachs147-1 (top left), LfL07 (top right), Rhy174 (bottom left) and Rhy017 (bottom right). Mean values of replicates 1 and 2 are displayed.

Table 3.3-6: Number of DH lines, mean disease scores of the experiment and disease scores for the parents; values represent the means of both replicates.

Isolate	n=	Population mean	Clho3515	Alexis
Sachs147-1	238	0.82	0	4
LfL07	237	1.57	0	4
Rhy174	236	2.60	0.44	3.98
Rhy017	239	1.39	0	4

As demonstrated, the response of this DH population to the four scald isolates was highly differential. The experiment with Sachs147-1 is characterized by an unproportionally high number of fully resistant lines and very few lines with an intermediate reaction. Infection with isolate LfL07 as well resulted in a high number of resistant lines, but in this case a considerable number of DH lines are not fully resistant to scald. These lines therefore developed a few symptoms with disease scores ranging from 1 to 2.5. Inoculation with isolate Rhy174 left hardly any DH lines without disease symptoms. A high number of lines displayed medium resistance with scores between 1 and 2, and about half of the DH lines were fully susceptible with a score of 4. The distribution resulting from the assessment with isolate Rhy017 is quite similar with a clear differentiation between fully resistant and susceptible lines, but fewer resistant lines than in the Sachs147-1 experiment and accordingly more susceptible lines.

The different reaction and the skewness of the distributions suggest that the putative resistance loci identified in chapter 3.2 provide a differential and independent effectiveness against the respective isolates. In order to evaluate this possibility the X^2 test was employed to test the five hypotheses described in chapter 3.1. X^2 values for the respective isolates and hypotheses are reported in Table 3.3-7.

For the experiment with Sachs147-1 H1 was accepted at alpha = 5%, suggesting that two loci are active and both fully effective. H2 was accepted for LfL07 at alpha = 10%, indicating one fully and one partially effective locus. With alpha = 10% and an extremely low X^2 test value H5 was accepted for Rhy174, implying that one locus is active conveying partial resistance. None of the five hypotheses was accepted for Rhy017. This result was unexpected as the distribution for Rhy017 was similar to the one for Sachs147-1, only less strongly skewed, implying the presence of at least one fully effective locus. Taking into consideration the possibility that segregation of the population might be distorted in the region of one of the loci, another hypothesis was tested, assuming one fully effective locus with a segregation ratio of 3:2. This hypothesis was accepted at alpha = 10% with a X^2 test value of 1.96.

Table 3.3-7: X² test values for DH33349.

	Hypothesis	H1	H2	H3	H4	H5	H6[1]
Sachs 147-1	X² value	3.50	82.16	374.37	87.13	113.01	
	Significance	**	-	-	-	-	
LfL07	X² value	17.33	4.50	133.68	16.75	59.75	
	Significance	-	*	-	-	-	
Rhy174	X² value	133.99	81.25	92.34	5.49	0.02	
	Significance	-	-	-	***	*	
Rhy017	X² value	14.23	55.30	245.27	19.92	28.82	1.96
	Significance	-	-	-	-	-	*

*, **, *** hypothesis is accepted at alpha = 0.1, 0.05 or 0.01, respectively; - hypothesis is rejected on all significance levels.

[1]H6 = 1 resistance locus in the population conveying full resistance, segregating 3:2

3.3.2.2 Genetic mapping

Genetic maps were created for chromosomes 3H and 6H with focus on the chromosomal regions identified for the putative resistance loci in chapter 3.2. The resulting linkage group for chromosome 3H consists of 52 markers covering 72.9 cM. Chromosome 6H is represented by a linkage group with 40 markers covering 113.5 cM (see figure 3.3-13).

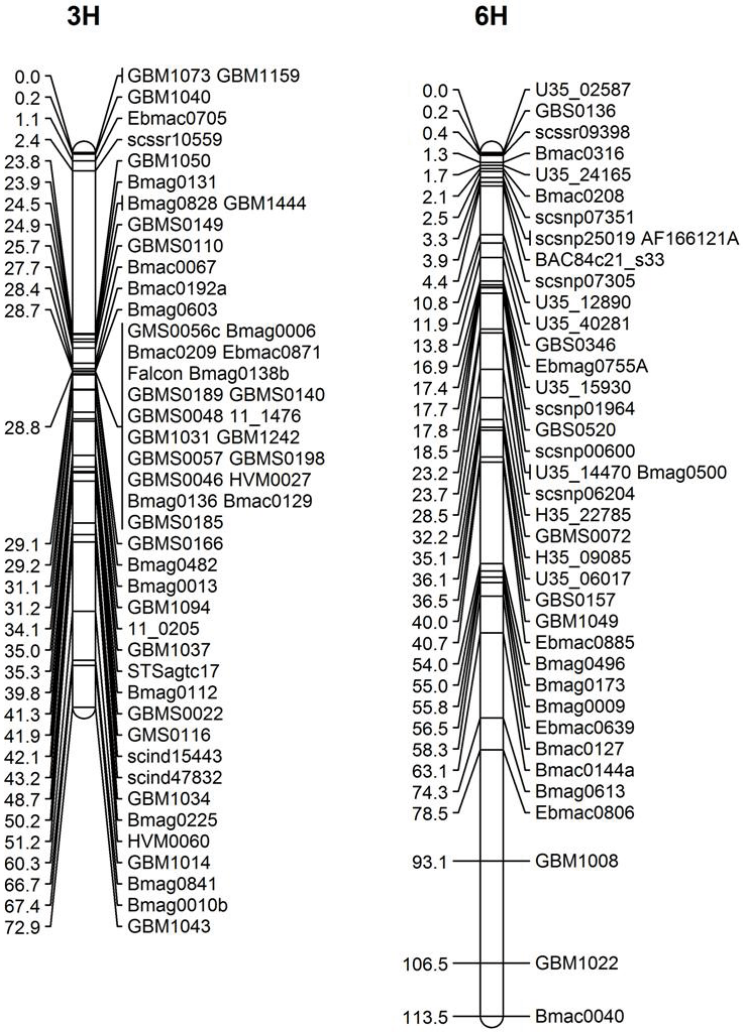

Figure 3.3-13: Linkage groups for barley chromosomes 3H and 6H mapped in DH population DH33349.

22 markers mapped into the region of interest for *Rrs*1 on chromosome 3H as defined in Hofmann et al. 2013 (figure 3.3-14), co-segregating markers being counted as one. The 22 markers covered a genetic distance of 26.7 cM with flanking markers being GBM1444 and HVM0060.

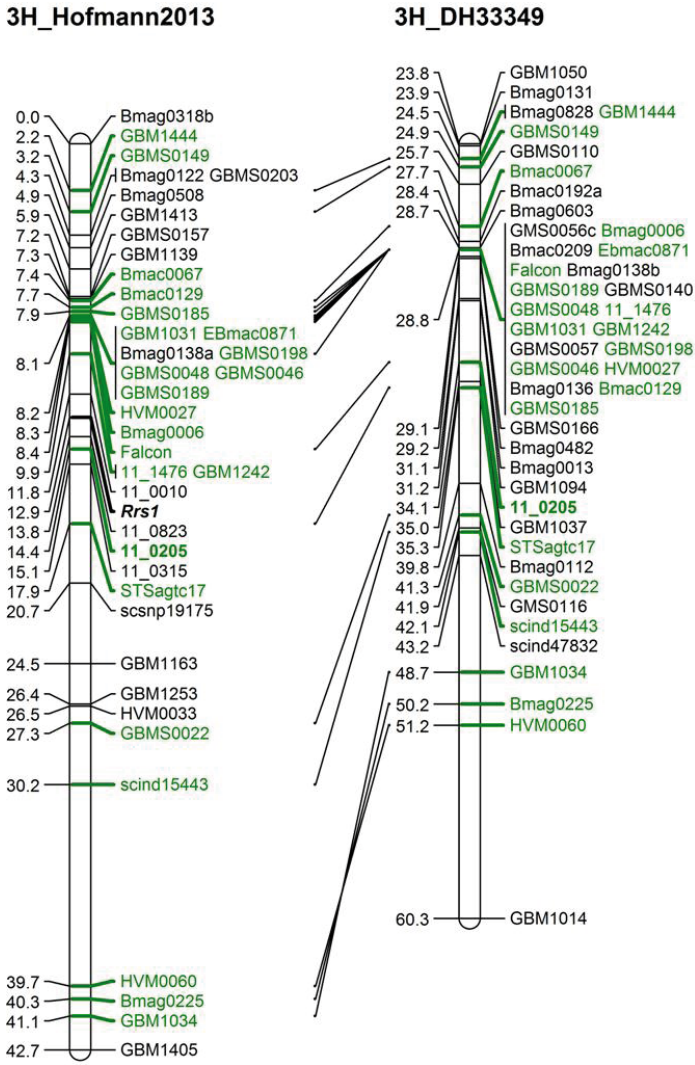

Figure 3.3-14: Map alignment. Left: consensus map of chromosome 3H as published in Hofmann et al. (2013) (see chapter 2.3). Right: centromeric region of chromosome 3H mapped in population DH33349 (see figure 3.3-13).

Based on the results from chapter 3.2 the region of interest for chromosome 6H was defined as the distal part of the short arm of chromosome 6H, the proximal flanking marker being Bmag0500. A total of 19 markers mapped into this region, co-segregating markers again being counted as one, covering a genetic distance of 23.2 cM (figure 3.3-15).

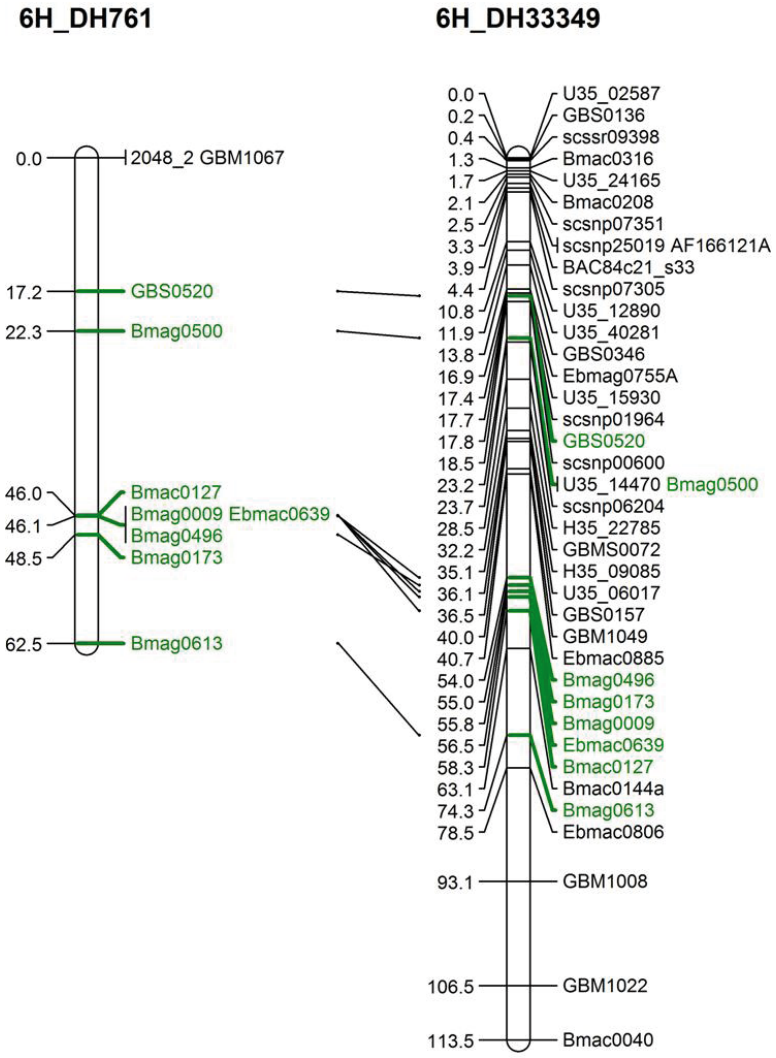

Figure 3.3-15: Map alignment. Left: chromosome 6H mapped in DH761 (see chapter 3.3.1.4). Right: chromosome 6H mapped in population DH33349 (see figure 3.3-13).

3.3.2.3 QTL analysis of the scald resistance reaction

The statistical analysis of the phenotypic data obtained in the greenhouse for DH33349 confirmed the presence of two resistance loci in CIho3515. In the experiments with scald isolates Sachs147-1 and LfL07 at least both loci contributed to the observed resistance. A QTL analysis was therefore considered to be the most efficient approach to confirm the loci identified in chapter 3.3.1, to verify the results from the statistical analysis (table 3.3-7), and in the case of the resistance locus on chromosome 6HS to narrow down further the region of the genome containing the resistance.

The QTL analysis was conducted using the linkage groups for chromosomes 3H and 6H mapped in DH33349 (figure 3.3-13) and the phenotypic data which is displayed in figure 3.3-12. A large number of markers co-segregated with markers Falcon, 11_1476, GBM1242 and HVM0027 on chromosome 3H. As those markers did not convey additional information, they were excluded from all QTL map charts to achieve better comprehensibility.

The first analysis of chromosome 3H resulted in a QTL with two pronounced peaks within 6 cM and a significant decrease of the LOD score in between (figure 3.3-16). Comparison of the linkage map with published consensus maps (Varshney et al. 2007, Ramsay et al. 2000) revealed that SSR markers Bmag0013 and GBM1037 usually map into the distal region of the long arm of chromosome 3H. Bmag0013 and GBM1037 were therefore excluded from all further QTL analyses.

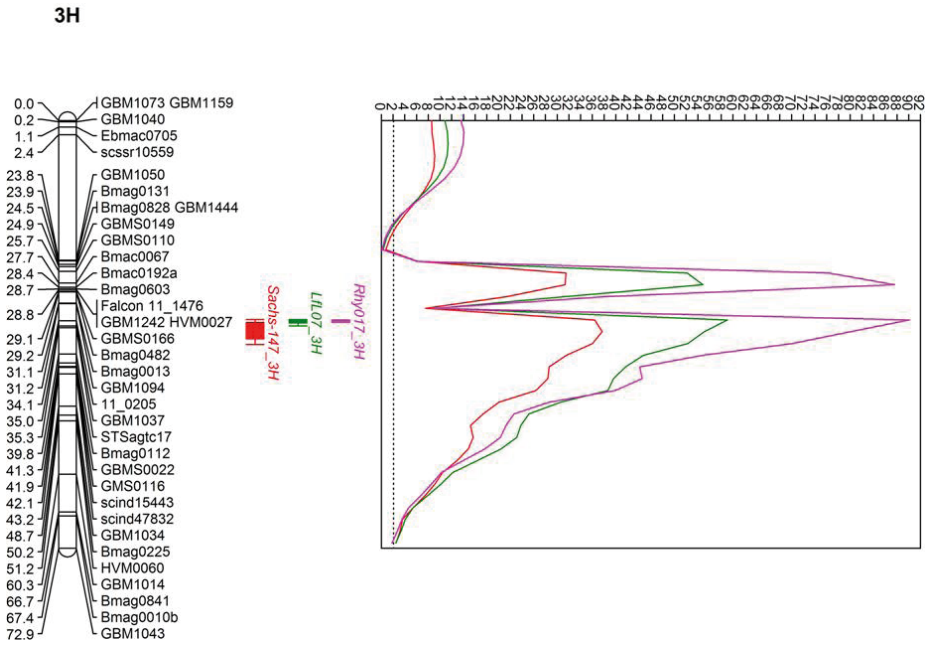

Figure 3.3-16: QTL on chromosome 3H of DH33349 for the scald isolates Sachs147-1 (red), LfL07 (green) and Rhy017 (pink). The LOD threshold for a significant QTL is indicated by a dotted black line. QTL analysis was conducted without cofactors.

The analysis was repeated for chromosomes 3H and 6H, and six (3H) and two (6H) QTL were identified. Analysis with the data for isolate Sachs147-1 revealed QTL for *R. commune* resistance on both chromosomes, whereas isolates Rhy017 and LfL07 caused two QTL each on chromosome 3H, and analysis with the Rhy174 data resulted in one QTL on chromosome 6H. LOD scores, R^2 % and additive effects for these QTL are reported in table 3.3-8. The QTL positions and LOD curves are presented in figure 3.3-17.

Table 3.3-8: QTL detected in DH33349 after inoculation with isolates Sachs147-1, LfL07, Rhy174 and Rhy017. **No cofactors** were used in the analysis. The location of the QTL in the genome is presented in figure 3.3-17.

QTL	LOD score	R^2 %	Additive effect
Sachs147-1_3Ha	9.09	16.1	-0.673
Sachs147-1_3Hb	38.12	52.2	-1.079
Sachs147-1_6H	5.54	10.2	-0.468
LfL07_3Ha	11.40	19.9	-0.734
LfL07_3Hb	62.53	70.3	-1.370
Rhy174_6H	53.97	65.1	-1.195
Rhy017_3Ha	14.11	23.8	-0.880
Rhy017_3Hb	106.92	87.3	-1.789

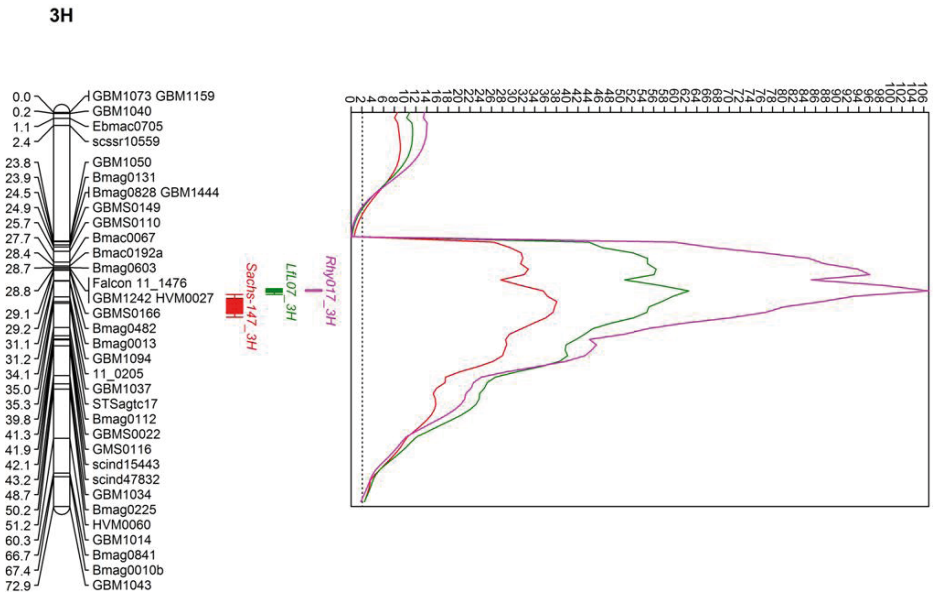

Figure 3.3-17a

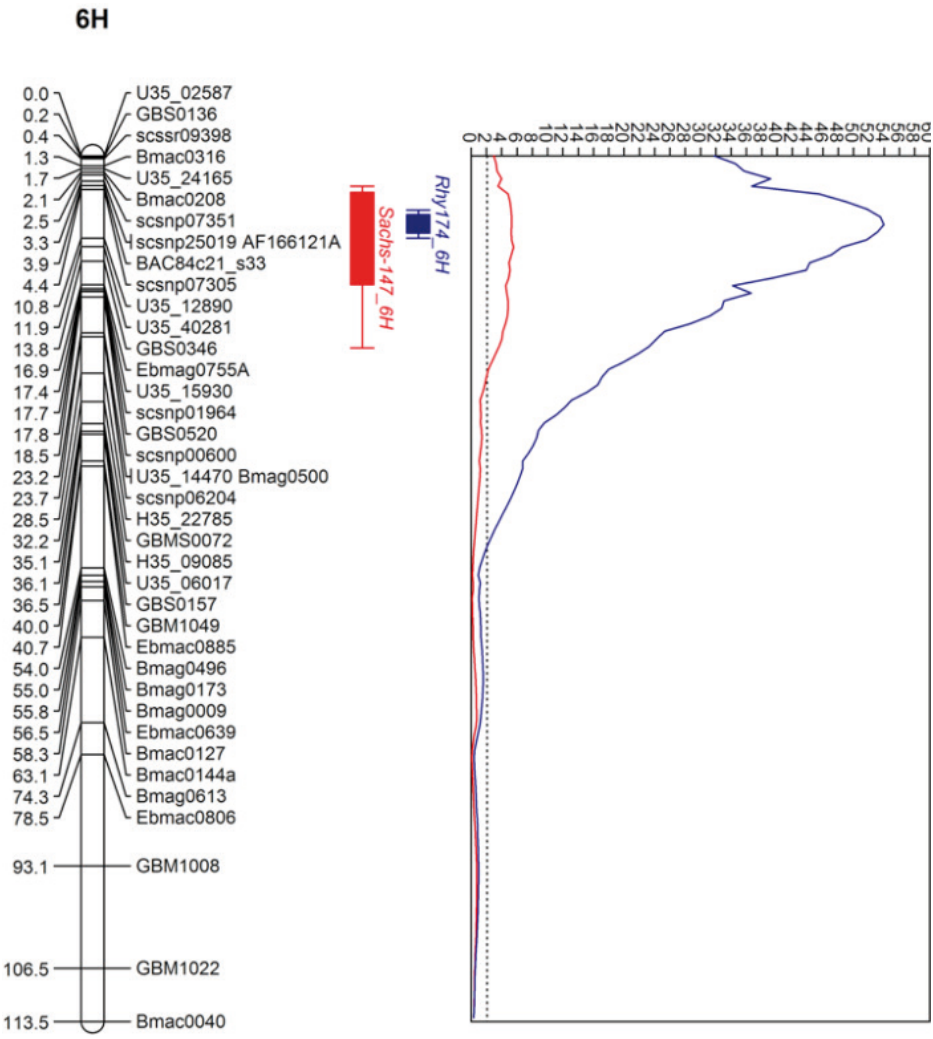

Figure 3.3-17b

Figure 3.3-17: QTL for chromosomes 3H (a) and 6H (b) of DH33349 for isolates Sachs147-1 (red), LfL07 (green), Rhy174 (blue) and Rhy017 (pink). The LOD threshold for a significant QTL is indicated by a dotted black line. QTL were calculated without cofactors.

In addition, a further QTL analysis was conducted using markers 11_0205 and scsnp07305 as cofactors for chromosomes 3H and 6H respectively. 11_0205 was selected for being the closest marker to the *Rrs*1 resistance locus from Hofmann et al. (2013) which was mapped in DH33349 as well (figure 3.3-14). Marker scsnp07305 was selected based on the results from the QTL analysis of DH761 (see chapter 3.3.1.5) and the alignment of chromosomes 6H from populations DH761 and DH33349 (figure 3.3-15). The marker was estimated to be the one mapping closest to the putative location of the QTL.

Analysis using these markers as cofactors returned six QTL on chromosome 3H, two for each LfL07 and Rhy017 and one for each Sachs147-1 and Rhy174, and three QTL on chromosome 6H, one for each isolate except Rhy017. LOD scores, R^2 % and additive effects are given in table t5. The QTL positions and LOD curves are presented in figure 3.3-9.

Table 3.3-9: QTL detected in DH33349 after inoculation with isolates Sachs147-1, LfL07, Rhy174 and Rhy017. Markers **11_0205 and scsnp07305 were used as cofactors** in the analysis. The location of the QTL in the genome is presented in figure 3.3-18.

QTL	LOD score	R^2 %	Additive effect
Sachs147-1_3H	45.90	58.9	-1.095
Sachs147-1_6H	14.12	23.9	-0.520
LfL07_3Ha	68.66	73.7	-1.385
LfL07_3Hb	3.01	5.7	-0.355
LfL07_6H	7.92	14.3	-0.345
Rhy174_3H	3.66	6.9	-0.247
Rhy174_6H	57.47	67.4	-1.202
Rhy017_3Ha	3.26	6.1	-0.203
Rhy017_3Hb	106.81	87.2	-1.787

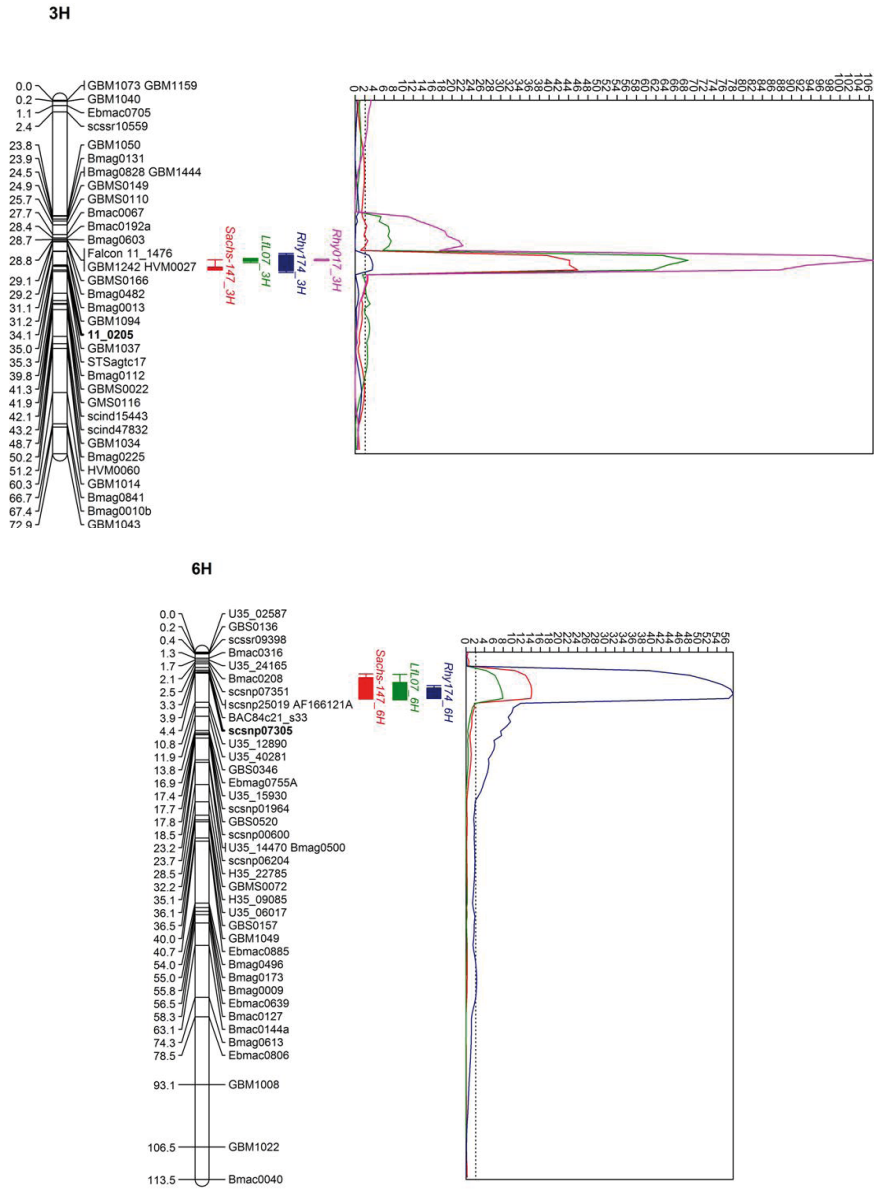

Figure 3.3-18: QTL on chromosomes 3H (top) and 6H (bottom) of DH33349 for isolates Sachs147-1 (red), LfL07 (green), Rhy174 (blue) and Rhy017 (pink). The LOD threshold for a significant QTL is indicated by a dotted black line. QTLs were calculated using markers 11_0205 and scsnp07305 as cofactors for chromosomes 3H and 6H respectively.

The results of the two QTL analyses confirm the findings from the chi² test. Two major loci contributed considerably to the resistance after infection with isolate Sachs147-1, one on chromosome 3H and one on chromosome 6H. The major resistance source on chromosome 3H was clearly the QTL located in the centromeric region of chromosome 3H, potentially an allele of the *Rrs*1 resistance gene. Whether the smaller QTL on chromosome 3H from the first analysis was attributed to (additional) environmental influence and/or experimental error or represents a true second resistance locus remains unclear at this point. But as the QTL disappeared when using cofactors in the analysis the first possibility is considered to be the more likely one.

The same reasoning was applied to the minor QTL on 3H from the analysis of the LfL07 data. The main resistance source was identified as the major QTL on chromosome 3H, with the QTL on chromosome 6H contributing to a lesser degree.

The dominating source for the resistance observed in the assessment with Rhy174 was the QTL on chromosome 6H. Though a QTL was also detected on chromosome 3H after including cofactors in the analysis, this QTL contributed only in a very small part to the phenotypic variance, confirming the hypothesis that one locus, the less effective one, was the source of the observed resistance in this material.

Analysis of the Rhy017 experiments only returned two QTL on chromosome 3H with and without cofactors. In both cases a small QTL could be observed again on the distal part of the short arm of this chromosome, but the same rationale as before is considered acceptable and these minor QTL are therefore categorized as negligible.

The genomic location of the observed QTL was very consistent over all experiments and confirmed the findings of the work described in chapter 3.2. Two resistance loci are present in CIho3515. One is located in the centromeric region of chromosome 3H where the Rrs1 scald resistance gene has repeatedly been mapped. This gene is on average the more effective one, contributing between 55% and 85% of the observed resistance in three of the four experiments. Only against isolate Rhy174 a very low efficacy of 7% was observed. The other locus, although on average less effective than the first one, nevertheless contributed considerably to the observed resistance in three of the four experiments. In the experiment with isolate Rhy174 it was the only effective locus. The QTL for this locus were consistently detected on the short arm on chromosome 6H.

Based on the assumption that one locus only was responsible each time for the observed resistance against isolates Rhy017 and Rhy174, an attempt was made to map these two resistance genes as phenotypic markers. Two approaches were followed: Approach A assigned the resistant genotype “b” to all DH lines with a disease score smaller than 2.00, whereas all DH lines with a disease score of 2.00 or higher were assigned to the susceptible genotype “a”. Approach B assigned the resistant genotype “b” to all DH lines with a disease score smaller than the population mean, 1.39 and 2.60 for Rhy017 and Rhy174 respectively, whereas all DH lines with a disease score equal to that population mean or higher were assigned to the susceptible genotype “a”. The loci were designated Rrs_{Rhy017} and Rrs_{Rhy174}. The resulting linkage maps are displayed in figure 3.3-19 and figure 3.3-20.

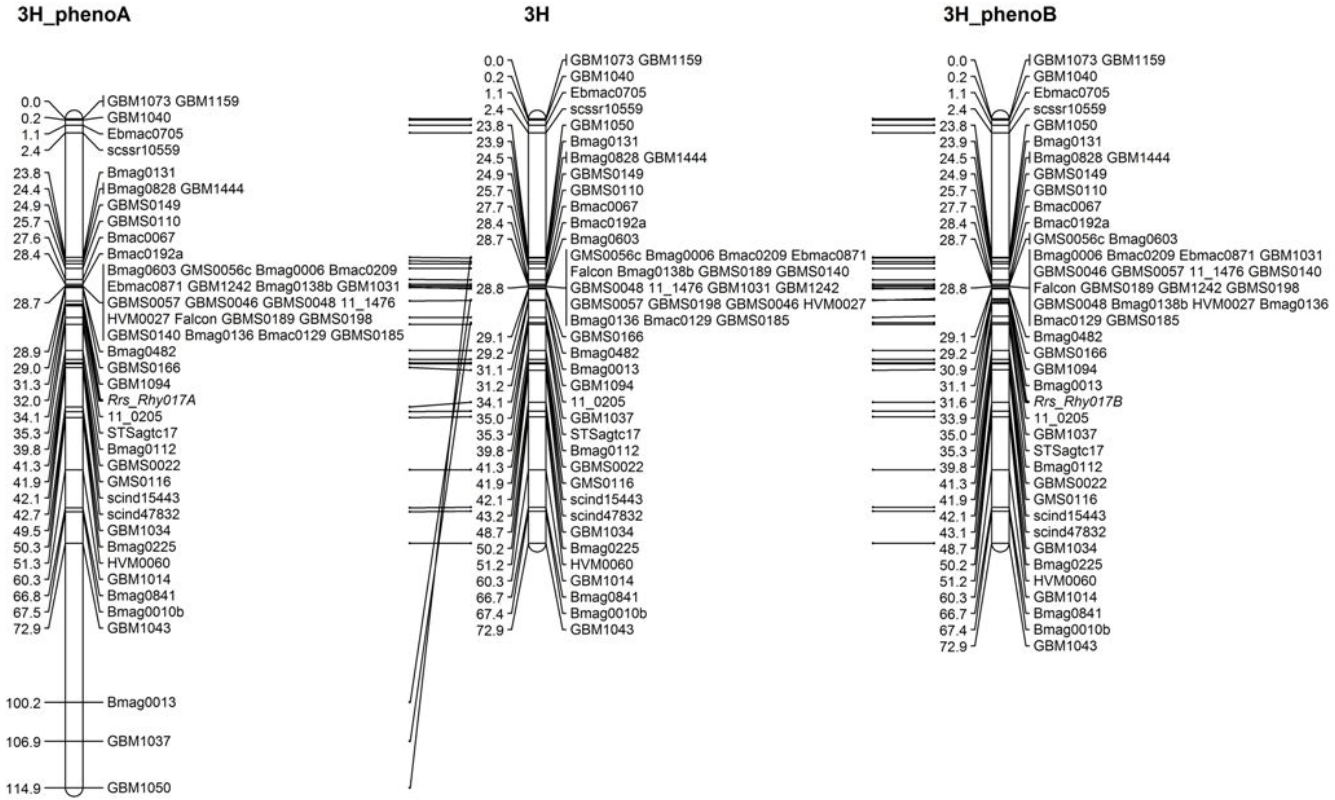

Figure 3.3-19: Linkage maps for chromosome 3H mapped in population DH33349. Left: phenotypic marker *Rrs*$_{Rhy017}$ mapped using approach A. Center: no phenotypic marker mapped. Right: phenotypic marker *Rrs*$_{Rhy017}$ mapped using approach B.

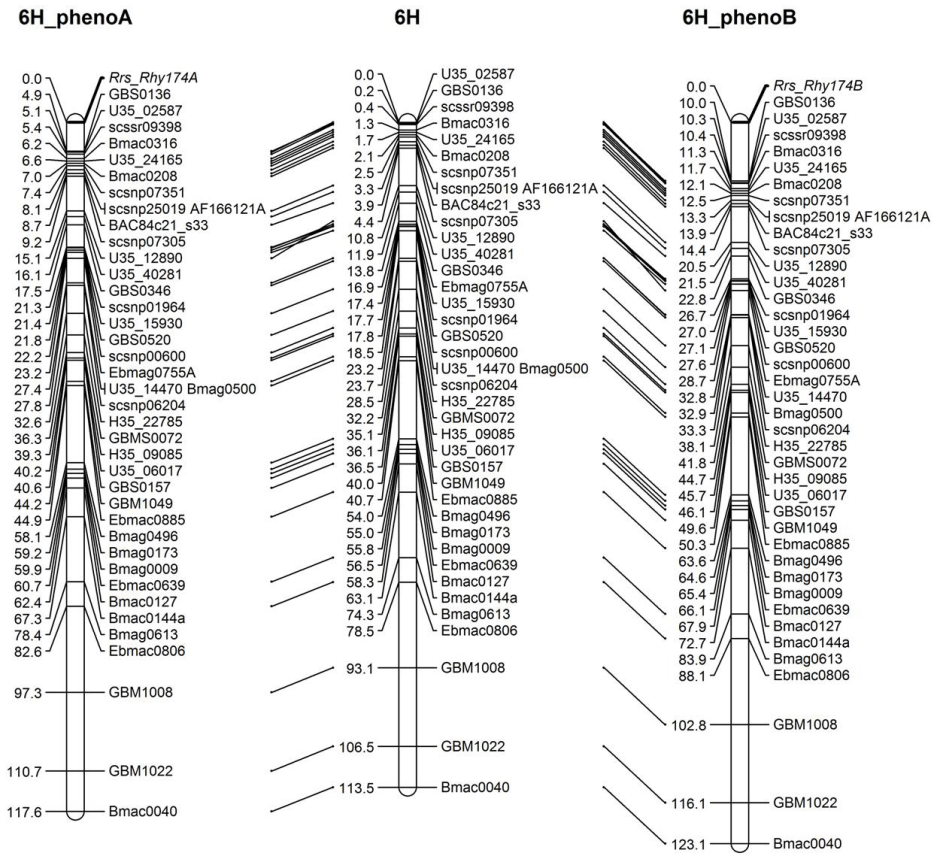

Figure 3.3-20: Linkage maps for chromosome 6H mapped in population DH33349. Left: phenotypic marker *Rrs*$_{Rhy174}$ mapped using approach A. Center: no phenotypic marker mapped. Right: phenotypic marker *Rrs*$_{Rhy174}$ mapped using approach B.

Rrs$_{Rhy017}$ maps between markers GBM1094 and 11_0205 using approach A (figure 3.3-19 left). The marker order using approach B is GBM1094-Bmag0013- *Rrs*$_{Rhy017}$-11_0205 (figure 3.3-19 right). SSR markers GBM1037 and Bmag0013, which, as already described, mapped into the wrong position in the original map (figure 3.3-13), have moved to the distal end of the long arm of chromosome 3H in the map constructed with approach A. The same can be observed for marker GBM1050.

Rrs$_{Rhy174}$ maps onto the distal end of chromosome 6HS using both approaches (figure 3.3-20). The distance to the next marker is with 10 cM twice as big using approach B compared to approach A with 4.9 cM. Including this phenotypic marker in the map of chromosome 6H has only minor effect on the order in which the markers map onto the chromosome (figure 3.3-20).

QTL analyses were repeated as already described using the linkage maps resulting from both approaches. LOD scores, R^2 % and additive effects are summarized for each analysis in tables 3.3-10 to 3.3-13, whereas QTL positions and LOD curves are presented in figures 3.3-21 to 3.3-24. The effect of *Rrs*$_{Rhy174}$ on the calculated QTL is very small. *Rrs*$_{Rhy017}$ on the other hand has considerable influence on the QTL detected on chromosome 6H. The observed effect is comparable between approach A and approach B and most prominent in the analysis of the data from isolate Rhy174. The QTL already detected is still visible, but an additional QTL with considerably higher LOD scores appears at the very end of the chromosome arm. LOD scores decrease considerably between the QTL, an effect that is strongly enhanced by the inclusion of cofactors in the analysis. A further effect of the cofactor is, that while the original, more proximal QTL is slightly enhanced, the new distal QTL is significantly reduced.

Table 3.3-10: QTL detected in DH33349 after inoculation with isolates Sachs147-1, LfL07, Rhy174 and Rhy017. Putative resistance loci *Rrs*$_{Rhy017}$ and *Rrs*$_{Rhy174}$ were mapped as phenotypic markers as described in **approach A**. **No cofactors** were used in the analysis. The location of the QTL in the genome is presented in figure 3.3-21.

QTL	LOD score	R^2 %	Additive effect
Sachs147-1_3H	40.84	54.6	-1.103
Sachs147-1_6Ha	8.82	15.7	-0.561
Sachs147-1_6Hb	5.54	10.2	-0.469
LfL07_3Ha	69.26	74.0	-1.388
LfL07_3Hb	40.07	54.1	-1.327
LfL07_6H	5.59	10.3	-0.492
Rhy174_6H a	106.70	87.5	-1.295
Rhy174_6H b	53.91	65.1	-1.196
Rhy017_3H	164.61	95.8	-1.827

Table 3.3-11: QTL detected in DH33349 after inoculation with isolates Sachs147-1, LfL07, Rhy174 and Rhy017. Putative resistance loci Rrs_{Rhy017} and Rrs_{Rhy174} were mapped as phenotypic markers as described in **approach A**. Markers **11_0205 and scsnp07305 were used as cofactors** in the analysis. The location of the QTL in the genome is presented in figure 3.3-22.

QTL	LOD score	R^2 %	Additive effect
Sachs147-1_3H	51.14	62.8	-1.132
Sachs147-1_6H	14.14	23.9	-0.521
LfL07_3Ha	76.43	77.4	-1.402
LfL07_3Hb	3.00	5.7	-0.362
LfL07_6Ha	7.04	12.8	-0.441
LfL07_6Hb	7.95	14.3	-0.343
Rhy174_3H	3.83	7.2	-0.257
Rhy174_6Ha	67.46	73.2	-1.146
Rhy174_6Hb	57.40	67.4	-1.204
Rhy017_3H	141.14	93.4	-1.828

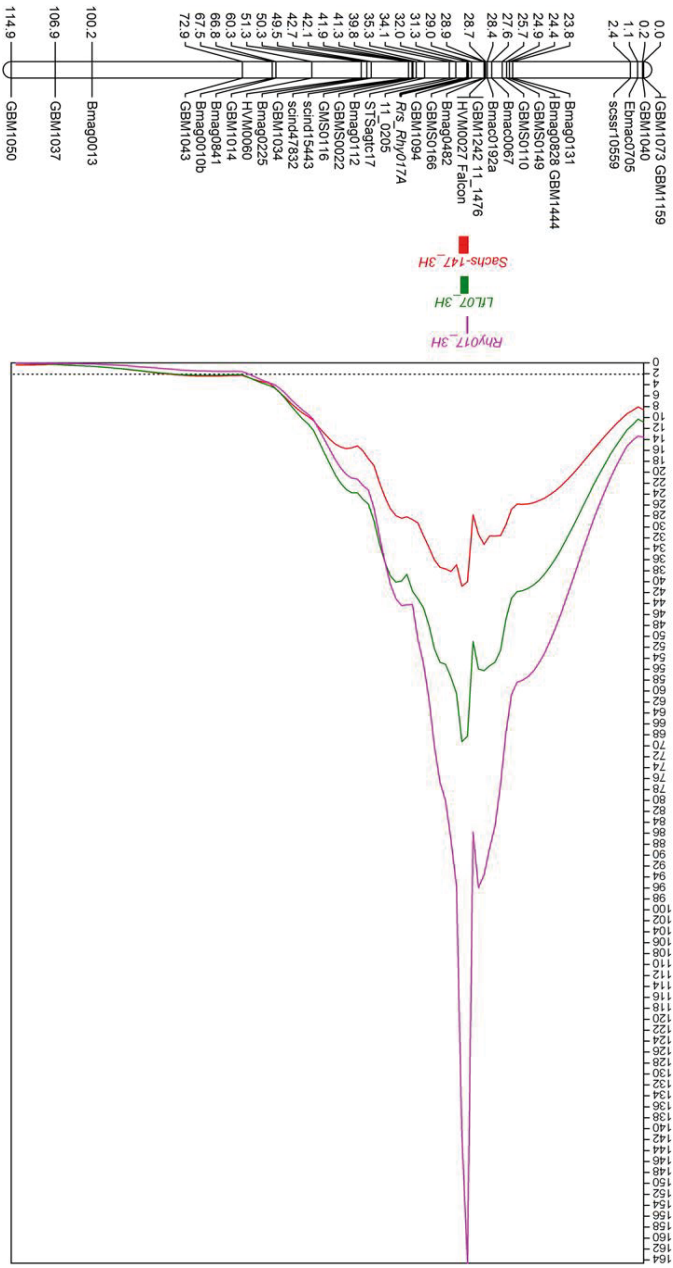

Figure 3.3-21a

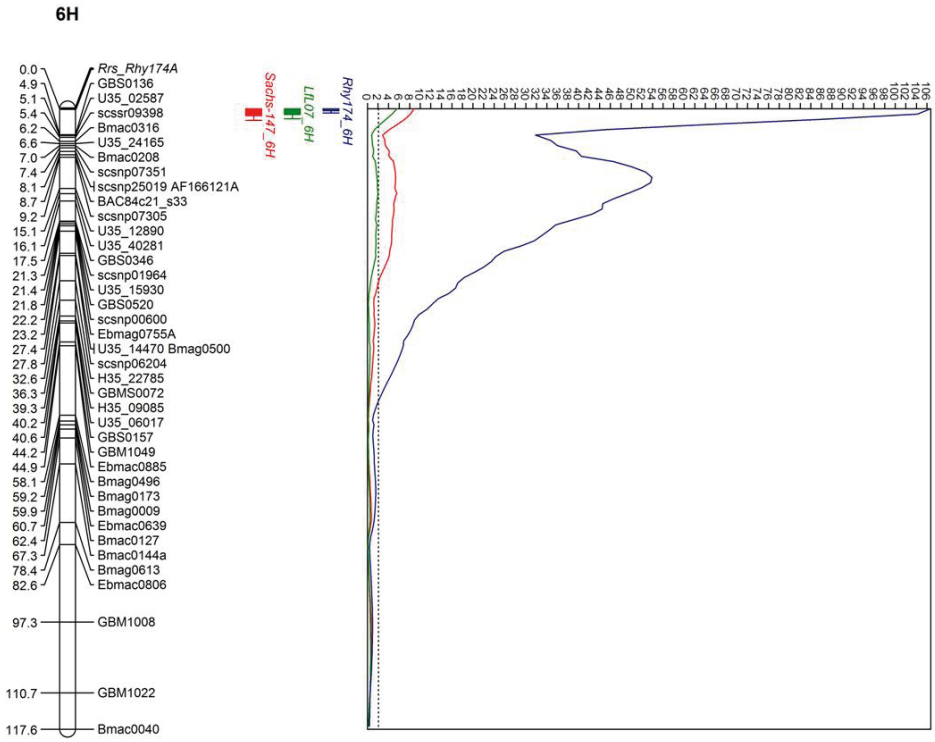

Figure 3.3-21b

Figure 3.3-21: QTL for chromosomes 3H (a) and 6H (b) identified in population DH33349 for isolates Sachs147-1 (red), LfL07 (green), Rhy174 (blue) and Rhy017 (pink). The LOD threshold for a significant QTL is indicated by a dotted black line. QTL were calculated **without cofactors**. Putative resistance loci Rrs_{Rhy017} and Rrs_{Rhy174} were mapped as phenotypic markers as described in **approach A**.

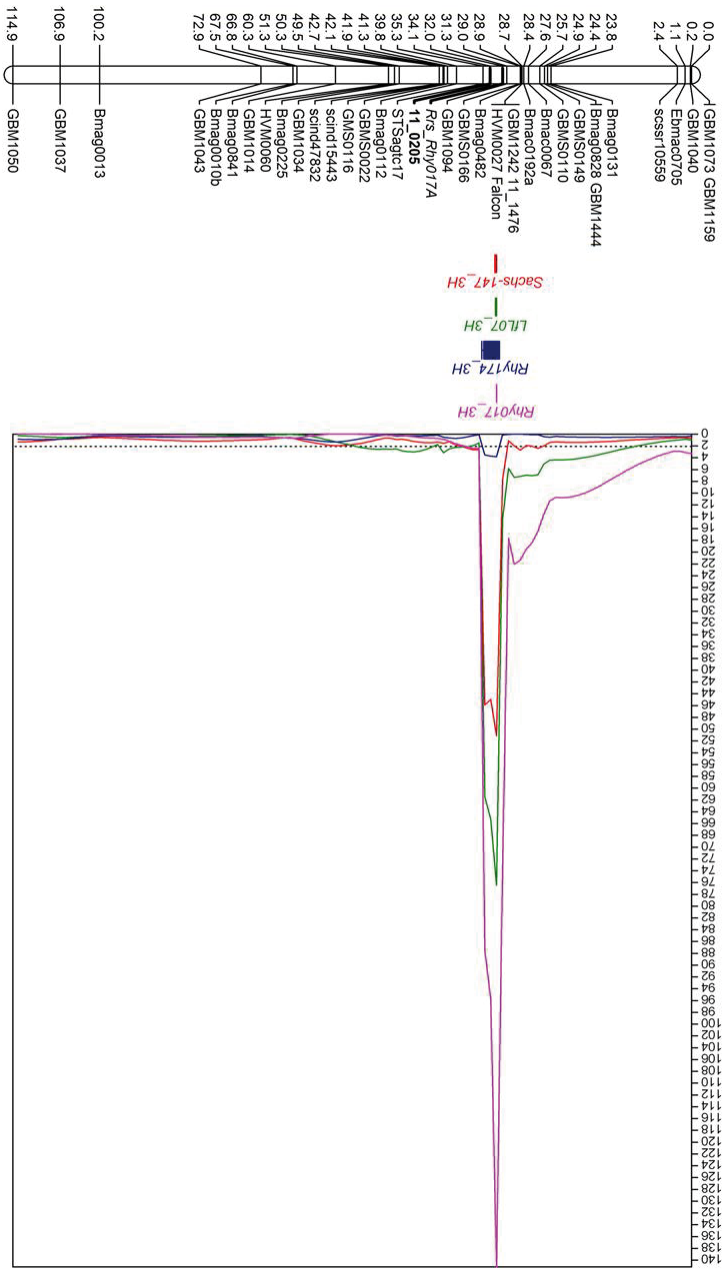

Figure 3.3-22a

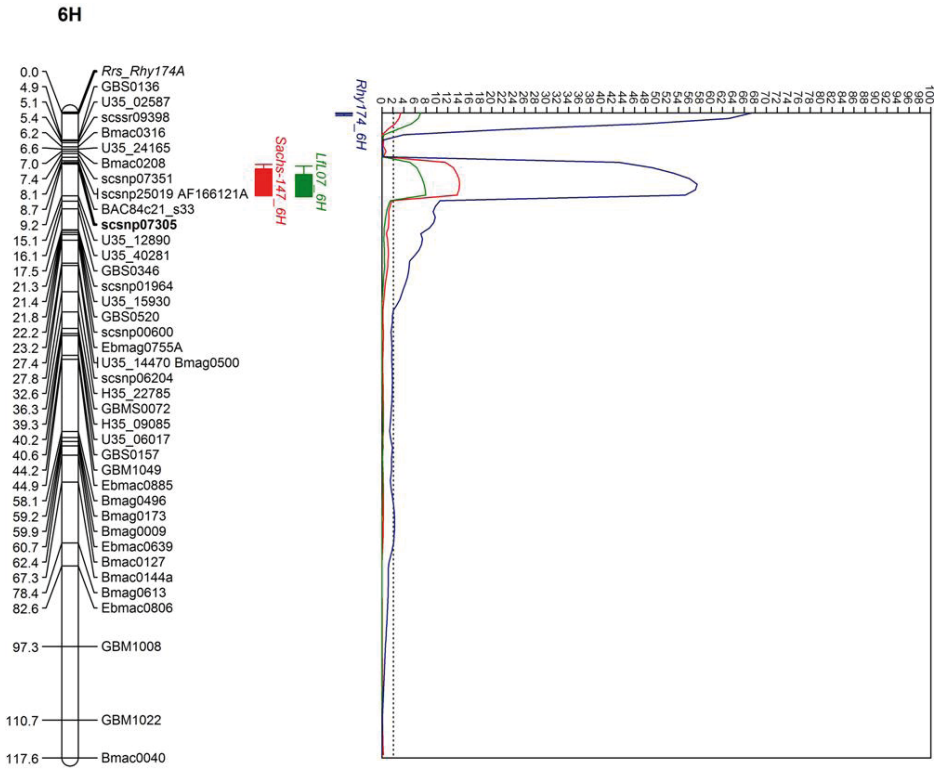

Figure 3.3-22b

Figure 3.3-22: QTL for chromosomes 3H (a) and 6H (b) identified in population DH33349 for isolates Sachs147-1 (red), LfL07 (green), Rhy174 (blue) and Rhy017 (pink). The LOD threshold for a significant QTL is indicated by a dotted black line. QTLs were calculated using **markers 11_0205 and scsnp07305 as cofactors** for chromosomes 3H and 6H respectively. Putative resistance loci Rrs_{Rhy017} and Rrs_{Rhy174} were mapped as phenotypic markers as described in **approach A**.

Table 3.3-12: QTL detected in DH33349 after inoculation with isolates Sachs147-1, LfL07, Rhy174 and Rhy017. Putative resistance loci Rrs_{Rhy017} and Rrs_{Rhy174} were mapped as phenotypic markers as described in **approach B**. **No cofactors** were used in the analysis. The location of the QTL in the genome is presented in figure 3.3-23.

QTL	LOD score	R^2 %	Additive effect
Sachs147-1_3Ha	9.09	16.1	-0.673
Sachs147-1_3Hb	41.08	54.8	-1.089
Sachs147-1_6Ha	11.66	20.3	-0.632
Sachs147-1_6Hb	5.43	10.0	-0.467
LfL07_3Ha	11.40	19.9	-0.734
LfL07_3Hb	73.34	76.0	-1.386
LfL07_6H	8.47	15.2	-0.594
Rhy174_6Ha	131.81	92.4	-1.374
Rhy174_6Hb	53.87	65.1	-1.192
Rhy017_3Ha	14.11	23.8	-0.880
Rhy017_3Hb	150.83	94.5	-1.815

Table 3.3-13: QTL detected in DH33349 after inoculation with isolates Sachs147-1, LfL07, Rhy174 and Rhy017. Putative resistance loci Rrs_{Rhy017} and Rrs_{Rhy174} were mapped as phenotypic markers as described in **approach B**. Markers **11_0205 and scsnp07305 were used as cofactors** in the analysis. The location of the QTL in the genome is presented in figure 3.3-24.

QTL	LOD score	R^2 %	Additive effect
Sachs147-1_3H	52.22	63.6	-1.123
Sachs147-1_6Ha	4.23	7.9	-0.347
Sachs147-1_6Hb	14.13	23.9	-0.521
LfL07_3Ha	82.23	79.8	-1.404
LfL07_3Hb	3.01	5.7	-0.355
LfL07_6Ha	7.87	14.2	-0.424
LfL07_6Hb	7.94	14.3	-0.344
Rhy174_3H	3.83	7.2	-0.254
Rhy174_6Ha	91.62	83.3	-1.113
Rhy174_6Hb	57.36	67.4	-1.199
Rhy017_3Ha	3.26	6.1	-0.203
Rhy017_3Hb	150.67	94.5	-1.813

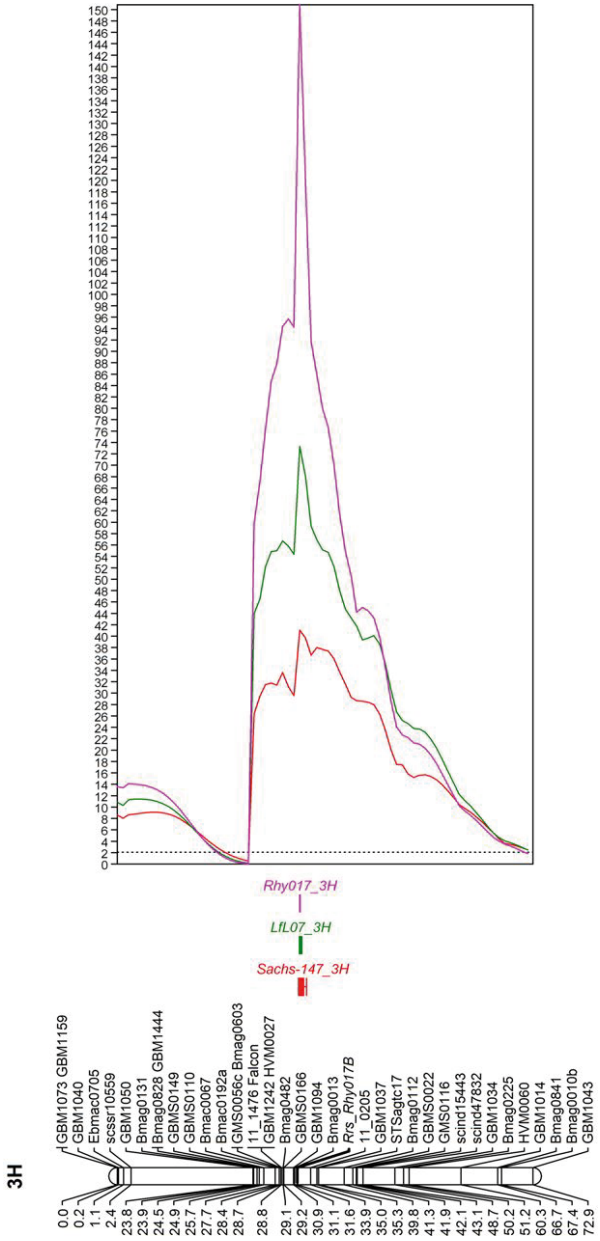

Figure 3.3-23a

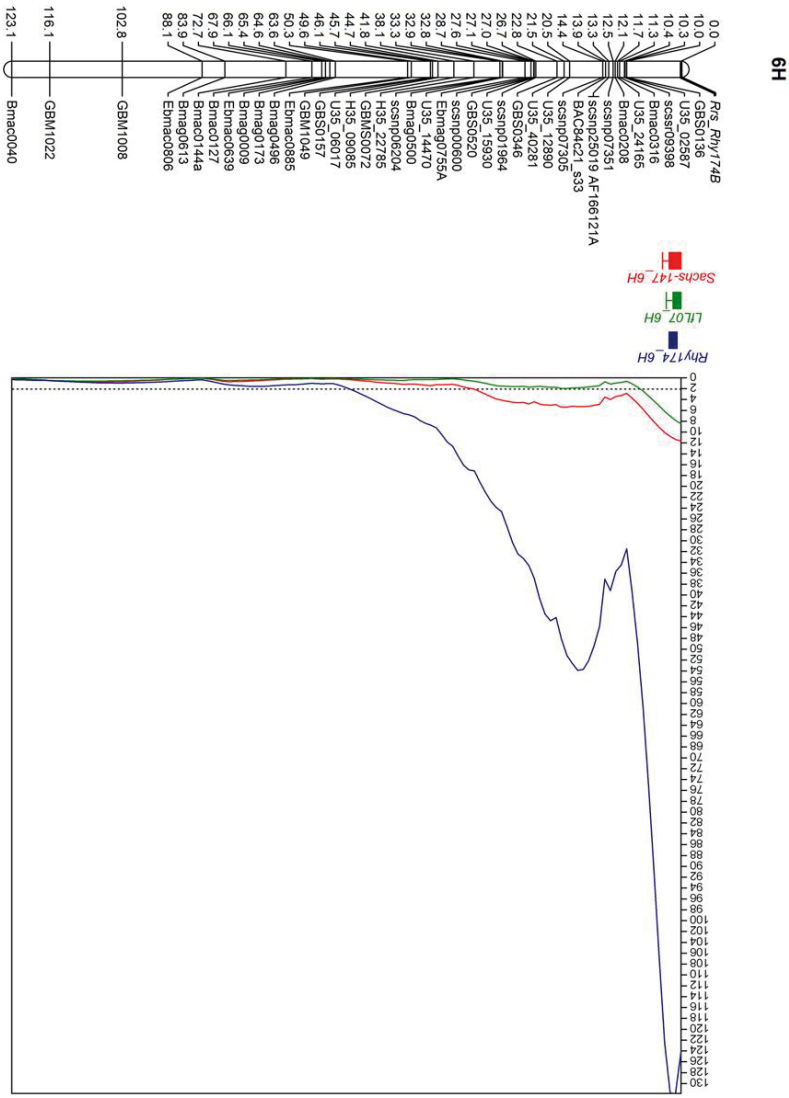

Figure 3.3-23b

Figure 3.3-23: QTL for chromosomes 3H (a) and 6H (b) identified in population DH33349 for isolates Sachs147-1 (red), LfL07 (green), Rhy174 (blue) and Rhy017 (pink). The LOD threshold for a significant QTL is indicated by a dotted black line. QTL were calculated **without cofactors**. Putative resistance loci Rrs_{Rhy017} and Rrs_{Rhy174} were mapped as phenotypic markers as described in **approach B**.

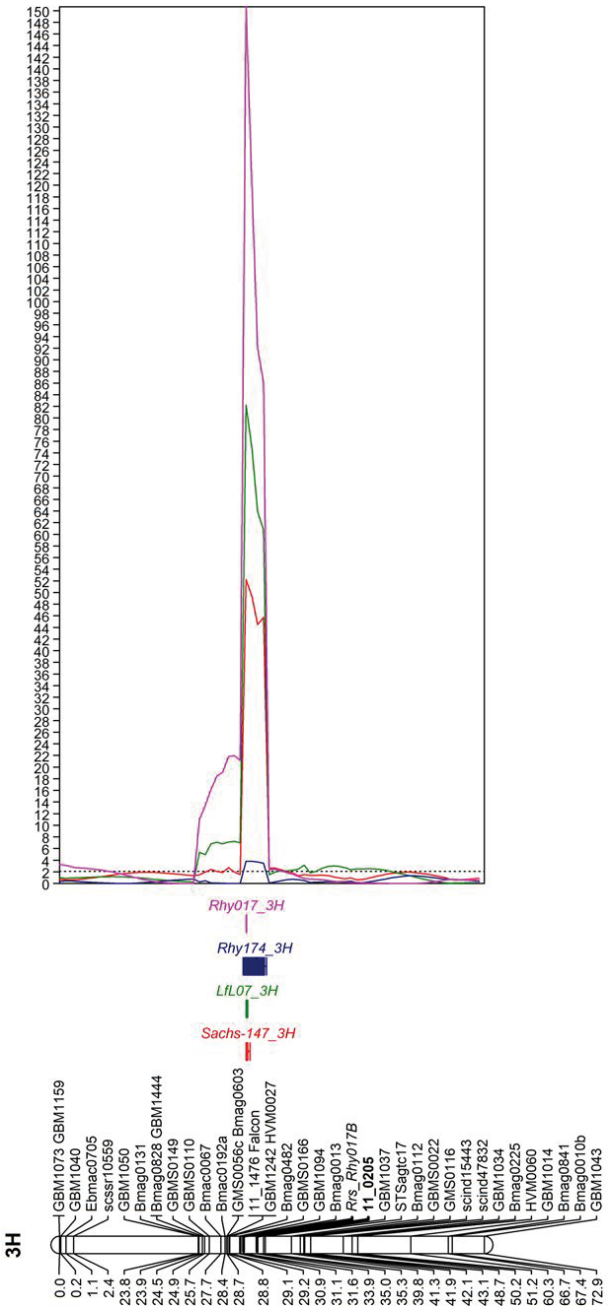

Figure 3.3-24a

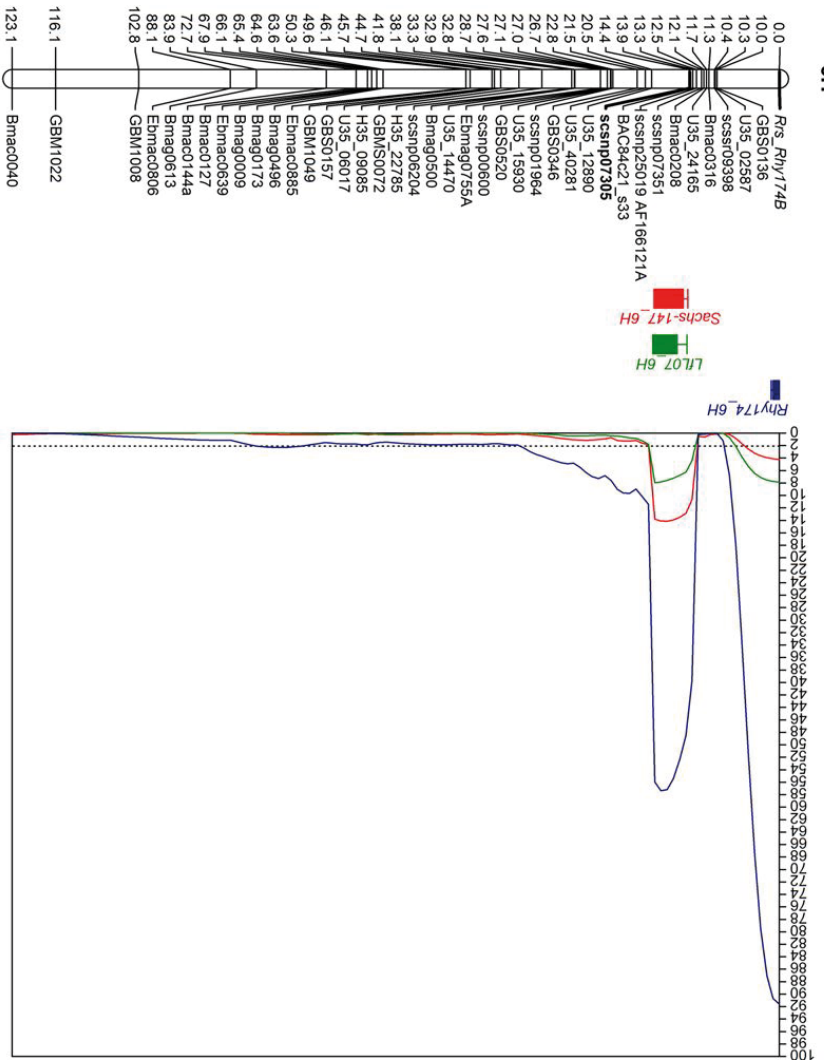

Figure 3.3-24b

Figure 3.3-24: QTL for chromosomes 3H (a) and 6H (b) identified in population DH33349 for isolates Sachs147-1 (red), LfL07 (green), Rhy174 (blue) and Rhy017 (pink). The LOD threshold for a significant QTL is indicated by a dotted black line. QTLs were calculated using markers **11_0205 and scsnp07305 as cofactors** for chromosomes 3H and 6H respectively. Putative resistance loci Rrs_{Rhy017} and Rrs_{Rhy174} were mapped as phenotypic markers as described in **approach B**.

3.3.2.4 Reaction of population DH33349 (CIho3515 x Alexis) to scald under field conditions

In order to evaluate the practical value of the resistance loci present in CIho3515 for barley breeding, population DH33349 was also assessed for scald resistance in the field with two fully randomized replications. The number of DH lines included in each replicate, the respective population means and the disease scores for the susceptible checks are reported in table 3.3-14.

Table 3.3-14: Number of DH lines, mean disease scores and disease scores for the susceptible check cv. Hendrix are given for replicates 1 and 2 and the overall mean.

Replicate	n=	Population mean	cv. Hendrix
1	229	2,00	5
2	176	1,86	5
Overall mean	230	1,95	5

The distribution of the DH lines to the disease scores are displayed in figure 3.3-25 for each replicate. Replicates 1 and 2 resulted in very similar distributions. The correlation of both replicates is presented in figure 3.3-26. The R^2 value of 0,72 was considered to be high enough to use the mean value of both replicates for the subsequent analysis. The distribution pattern of the population using the mean values from both replicates is presented in figure 3.3-27.

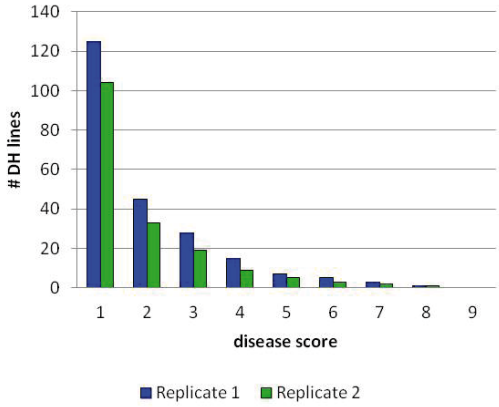

Figure 3.3-25: Response of DH33349 lines to field infection with scald. Blue bars represent replicate 1, green bars represent replicate 2.

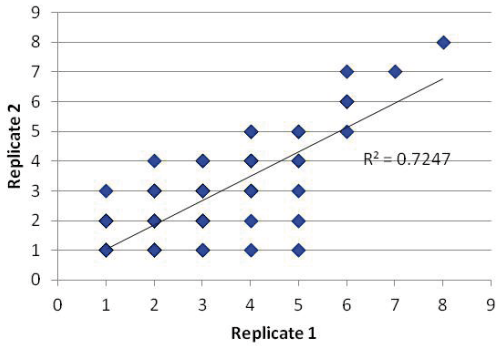

Figure 3.3-26: Disease scores for each DH line of replicate 1 are plotted against the respective disease scores of replicate 2. The resulting scatter plot displays the correlation of both replicates. The R^2 value of 0,72 indicates a high correlation between the replicates. Field scores were obtained in Freising, Bavaria/Germany.

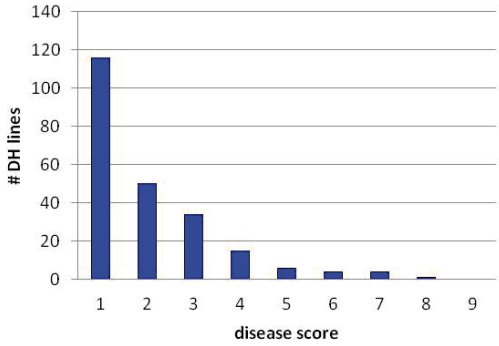

Figure 3.3-27: Response of DH33349 lines to field infection with scald. Mean values of replicates 1 and 2 are displayed.

QTL analyses were conducted again as already described with and without cofactors using the linkage maps without phenotypic markers. LOD scores, R^2 % and additive effects for each analysis are given in tabs 3.3-15 and 3.3-16. QTL positions and LOD curves are presented in figures 3.3-28 and 3.3-29. While the QTL are less defined, i.e. much broader and with lower LOD scores, they are still comparable with the QTL found in the greenhouse experiments. Both Rrs_{Rhy017} and Rrs_{Rhy174} are effective in the field.

Table 3.3-15: QTL detected in population DH33349 after scald infection in the field. No cofactors were used in the analysis. The location of the QTL in the genome is presented in figure 3.3-28.

QTL	LOD score	R^2%	Additive effect
field_3Ha	10.60	18.8	-0.605
field_3Hb	16.90	28.3	-0.804
field_6H	4.65	8.7	-0.429

Table 3.3-16: QTL detected in population DH33349 after scald infection in the field and using cofactors 11_0205 and scsnp07305. The location of the QTL in the genome is presented in figure 3.3-29.

QTL	LOD score	R^2%	Additive effect
field_3Ha	4.31	8.1	-0.504
field_3Hb	17.48	29.1	-0.781
field_6H	6.34	11.7	-0.435

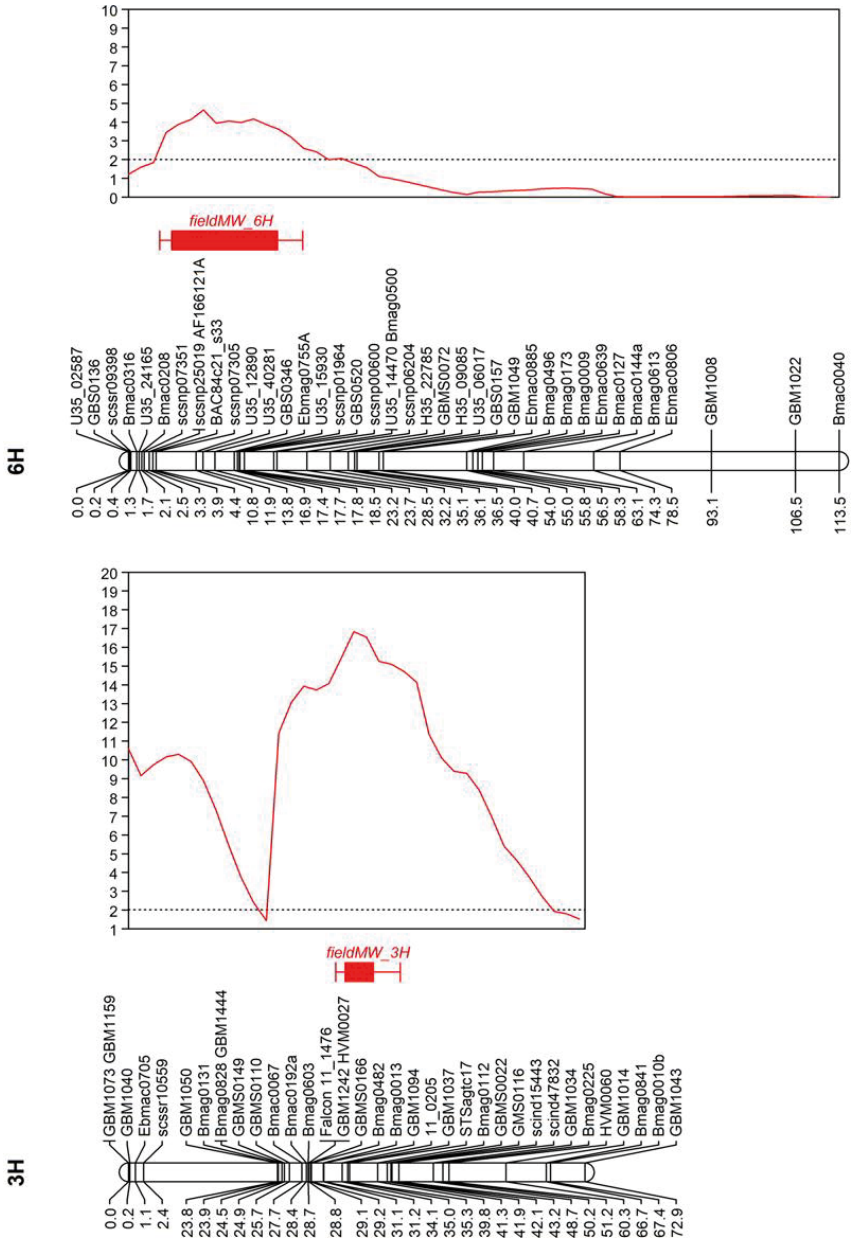

Figure 3.3-28: QTL for scald resistance on chromosomes 3H (left) and 6H (right) after field infection of population DH33349. The LOD threshold for a significant QTL is indicated by a dotted black line. QTL analysis was run without cofactors.

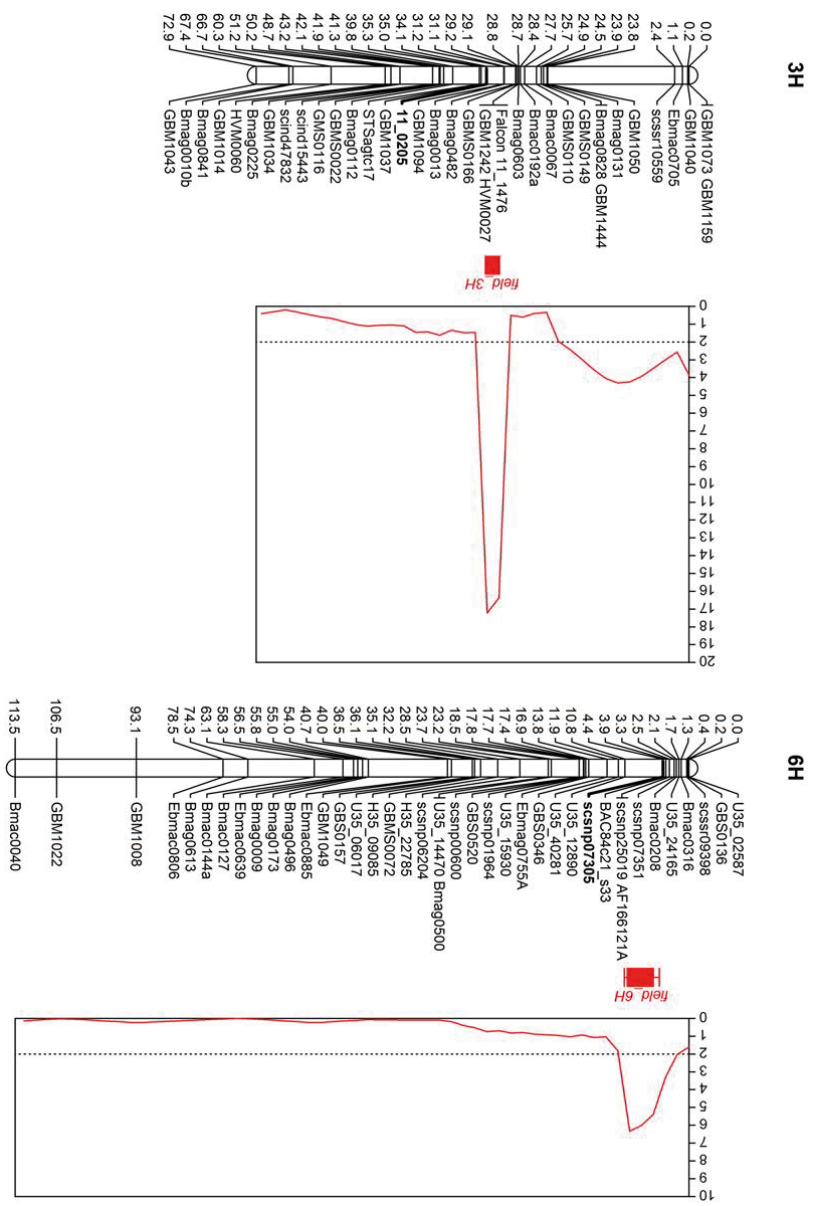

Figure 3.3-29: QTL for scald resistance on chromosomes 3H (left) and 6H (right) after field infection of population DH33349. The LOD threshold for a significant QTL is indicated by a dotted black line. QTL were calculated using markers 11_0205 and scsnp07305 as cofactors for chromosomes 3H and 6H, respectively.

3.3.2.5 Marker assisted selection

Marker assisted selection (MAS) was conducted to further determine the value of both resistance loci for breeding programs. Markers 11_0205 and scsnp07305 were used to select for Rrs_{Rhy017} and Rrs_{Rhy174} respectively. The data from the greenhouse tests with isolates Sachs147-1 and LfL07 was used as well as the field data.

DH lines were selected first with marker 11_0205, then with scsnp07305 only. In each case two subpopulations were created carrying only the CIho3515 or the Alexis allele of the respective marker (figs 3.3-30, 3.3-32 and 3.3-34). Additionally a MAS was conducted with both markers combined, thus creating four subpopulations with four different genotypes (Table 3.3-17, figs 3.3-31, 3.3-33 and 3.3-35)

Table 3.3-17: Possible genotypes in DH33349 after selection with markers 11_0205 and scsnp07305.

Genotype	11_0205 allele	scsnp07305 allele
b/b	CIho3515	CIho3515
b/a	CIho3515	Alexis
a/b	Alexis	CIho3515
a/a	Alexis	Alexis

This approach was applied to all three experiments used for the MAS attempt. The results from the MAS are displayed and summarized in figures 3.3-30 to 3.3-35 and table 3.3-18.

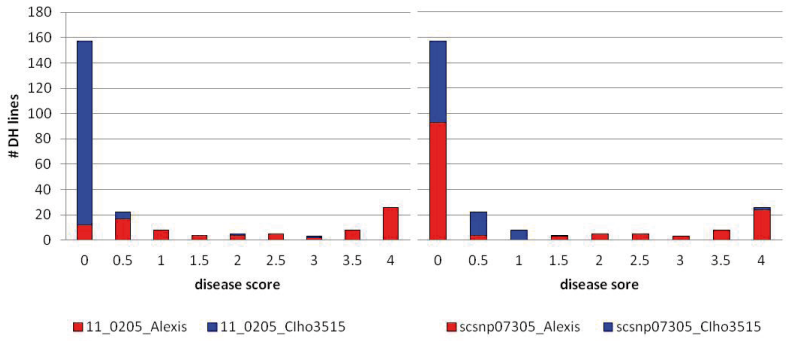

Figure 3.3-30: Response of DH33349 to infection with *R. commune* isolate Sachs147-1. Additionally lines were selected with markers 11_0205 (left) and scsnp07305 (right). Blue represents lines carrying the allele from the resistant parent Clho3515, red represents the lines carrying the allele from the susceptible parent Alexis.

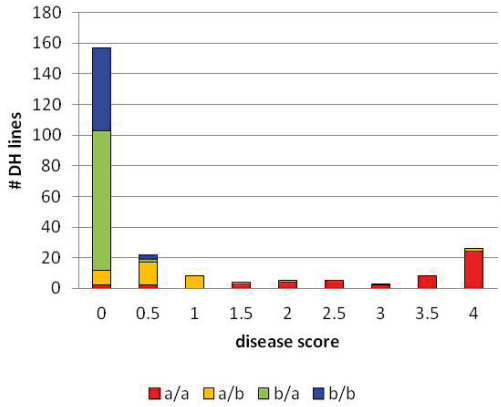

Figure 3.3-31: Response of DH33349 to infection with *R. commune* isolate Sachs147-1. Additionally lines were selected with markers 11_0205 and scsnp07305 combined. Blue, green, yellow and red represent genotypes b/b, b/a, a/b and a/a (see table 3.3-17), respectively.

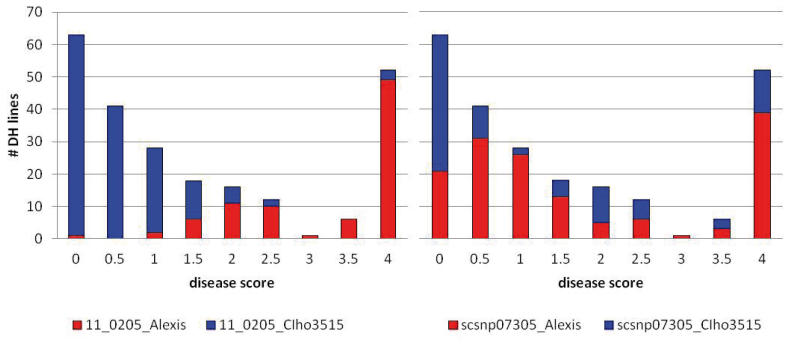

Figure 3.3-32: Response of DH33349 to infection with *R. commune* isolate LfL07. Additionally lines were selected with markers 11_0205 (left) and scsnp07305 (right). Blue represents lines carrying the allele from the resistant parent Clho3515, red represents the lines carrying the allele from the susceptible parent Alexis.

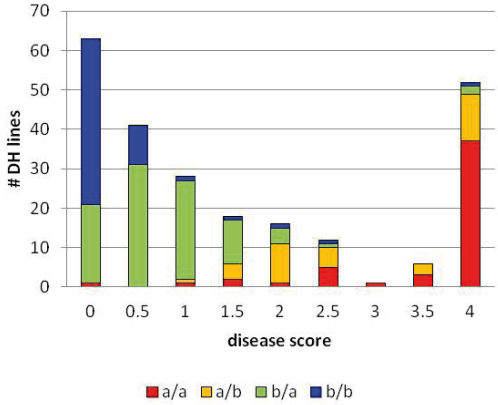

Figure 3.3-33: Response of DH33349 to infection with *R. commune* isolate LfL07. Additionally lines were selected with markers 11_0205 and scsnp07305 combined. Blue, green, yellow and red represent genotypes b/b, b/a, a/b and a/a (see table 3.3-17), respectively.

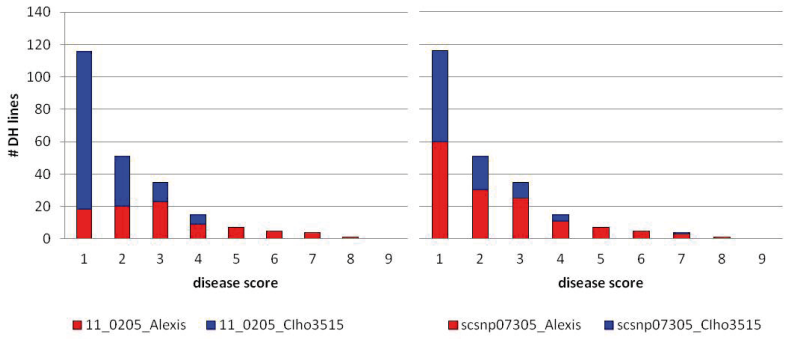

Figure 3.3-34: Response of DH33349 to infection with *R. commune* in the field. Additionally lines were selected with markers 11_0205 (left) and scsnp07305 (right). Blue represents lines carrying the allele from the resistant parent Clho3515, red represents the lines carrying the allele from the susceptible parent Alexis.

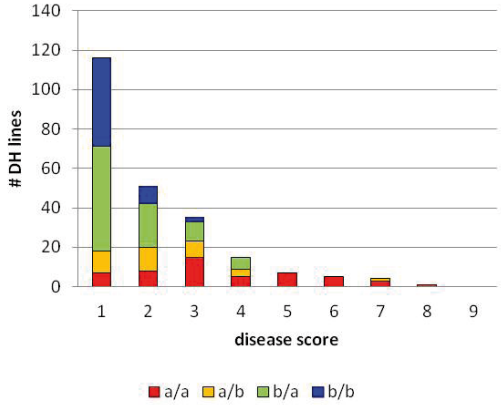

Figure 3.3-35: Response of DH33349 to infection with *R. commune* in the field. Additionally lines were selected with markers 11_0205 and scsnp07305 combined. Blue, green, yellow and red represent genotypes b/b, b/a, a/b and a/a (see table 3.3-17), respectively.

Table 3.3-18: Mean disease scores after inoculation with *R. commune* isolates Sachs147-1 and LfL07 and field infection for all sub-populations created through marker assisted selection.

Subpopulation	Mean disease score		
	Sachs147-1	LfL07	Field
11_0205_CIho3515	0.07	0.63	1.41
11_0205_Alexis	2.14	3.23	2.89
scsnp07305_CIho3515	0.30	1.27	1.54
scsnp07305_Alexis	1.07	1.77	2.23
11_0205/scsnp07305 b/b	0.03	0.31	1.18
11_0205/scsnp07305 b/a	0.10	0.82	1.55
11_0205/scsnp07305 a/b	0.74	2.82	2.11
11_0205/scsnp07305 a/a	3.15	3.52	3.43
11_0205/scsnp07305 {b/b & b/a & a/b}	0.20	1.04	1.55
11_0205/scsnp07305 {b/a & a/b & a/a}	1.07	1.97	2.21

3.4 Discussion of chapter B

CIho3515 has long been established as an accession highly resistant to *R. commune*. Numerous isolates of the pathogen from various geographies were used for assessment. In the work presented here, too, CIho3515 displayed outstanding resistance against five different isolates tested during the assessment of the mapping populations. In addition the accession has been used as a resistant reference in many experiments at the Bavarian State Research Center for Agriculture (LfL) and performed exceptionally against various isolates (Hofmann et al. 2013, Hofmann et al. unpublished, Schweizer et al. unpublished). These results confirm the findings reported in literature, and justify the selection of CIho3515 as a potential resistance donor for breeding programs.

3.4.1 Localization of scald resistance in the genome

To make resistance available for systematic knowledge-based breeding , the underlying genetics has to be explored. A DH population derived from a cross with scald susceptible cultivar Steffi, DH761, was already available at the LfL. Although with only 75 DH lines rather small, this population was considered sufficient for an initial screening. DH761 was therefore assessed for scald resistance in the greenhouse.

The resistance assessment resulted in a distribution which was strongly skewed to the left. A X^2 test confirmed the hypothesis that the resistance of CIho3515 is based two major loci. These findings are in line with what has been reported in various publications. Another consistent message from published reports concerns the identity of one of the two loci present in CIho3515. Although the nature of the *Rrs1* locus itself is still under debate, the agreed position is that CIho3515 is carrying an allele of this *Rrs1* locus. DH761 was therefore genotyped with microsatellite marker HVM0027, known to map into the centromeric region of chromosome 3H, where *Rrs1* is located. HVM0027 displayed very high correlation with the observed resistance, thereby supporting the hypothesis of Habgood and Hayes (1971), Starling (1971) and others.

DH761 was furthermore genotyped with STS marker ABG320, known to map distally on chromosome 7HS where the *Rrs2* scald resistance gene is located. The identity of the second locus present in CIho3515 has so far been a controversial issue. One theory that had been advanced is that the second locus of CIho3515 is identical to the one in

cultivars “Osiris” and “Atlas46” (Habgood & Hayes 1971). That locus has since been identified as *Rrs2* on the short arm of chromosome 7H (Hanemann et al. 2009). ABG320 however did not show any distinct correlation with the observed resistance, thereby refuting the hypothesis of Habgood & Hayes (1971) and supporting Starling (1971), who had excluded *Rrs2* as a possibility very early on.

The third marker to be used for this initial marker screening was the STS marker GemS13 (Schweizer et al. unpublished). GemS13 is associated with the resistance gene mapped in CIho8288 by Schweizer et al. (2004), $Rrs15_{CIho8288}$. The marker displays a rare allele in CIho8288 and a small number of other scald resistant barley accessions, one of these being CIho3515 (Schweizer et al. unpublished). This gave reason to expect that the second locus of CIho3515 might be an allele of $Rrs15_{CIho8288}$. However, GemS13 as well was not correlated with the observed resistance, ruling out $Rrs15_{CIho8288}$ as a potential resistance source of CIho3515 as well.

STS marker 2048_2, which was developed by means of an AFLP pool screening in a subpopulation of DH761 not carrying the *Rrs1* resistance gene, mapped on the short arm of chromosome 6H. 6HS is the putative location of *Rrs13*, a scald resistance gene located by Abbott et al. (1992) in a wild barley backcross. A number of QTL for scald resistance have also been identified on chromosome 6HS (Jensen et al. 2002, Cheong et al. 2006, Shtaya et al. 2006, Wagner et al. 2008). According to Cheong et al. (2006) these QTL represent a locus independent from *Rrs13*.

The QTL analysis conducted for validation purposes confirmed the presence of two loci present in CIho3515. One QTL was detected in the centromeric region of chromosome 3H. The highest LOD scores were detected between six and eight centiMorgan, close to cosegregating markers STSagtc17 and Falcon. These STS markers flank a 9.5 cM interval containing the *Rrs1* resistance gene in the consensus map published by Hofmann et al. (2013). The second QTL was detected on chromosome 6HS proximally of STS maker 2048_2. Due to the small number of DH lines and markers used, it was not possible to decide at this point whether the observed resistance locus corresponds to one on the loci already known on chromosome 6HS.

Due to the fact that population DH761 with only 75 DH lines was not suitable for a precise mapping attempt, a second DH population was generated from a cross between CIho3515 and Alexis.

3.4.2 Chromosome 3H locus

The linkage group constructed for chromosome 3H of population DH33349 at first glance seemed reasonably correct. Alignment with the consensus map of Hofmann et al. (2013) revealed a few minor inversions, and only very few markers in positions distinctly differing from what had previously been published. Only with the first QTL analysis it became apparent that the incorrect position of two of those markers, Bmag0013 and GBM1037, had a strong influence on the QTL analysis. According to Ramsay et al. (2000) and Varshney et al. (2007) both markers belong on the distal part of the long arm of chromosome 3H. Both markers were therefore excluded from all further QTL analyses.

The following analyses resulted in QTLs with considerable LOD scores whose peak was located in the centromeric region of chromosome 3H, where scald resistance gene *Rrs1* is located. Using marker 11_0205 (Hofmann et al. 2013) as cofactor further increased the LOD score and narrowed down the location of the QTL. It also removed the small second QTL observed distally on the short arm of chromosome 3H. This suggested that the resistance locus was indeed located close to 11_0205 and therefore *Rrs1*.

When calculating QTL for the experiment with isolate Rhy017 the QTL on 3H was the only one observed, implying that the resistance on chromosome 3H was the only effective one in this experiment. The resistance was consequently mapped as a phenotypic marker, Rrs_{Rhy017}. This marker mapped in a 5.4 cM (approach A) or 5.1 cM (approach B) interval flanked by markers 11_0205 and 11_1476. This correspond to the findings of Hofmann et al. (2013) again, who located the *Rrs1* gene in a 4.5 cM interval flanked by the same markers. It was therefore considered highly probable that Rrs_{Rhy017} is an allele of the *Rrs1* locus.

Mapping of Rrs_{Rhy017} using approach A also had some notable effects on the linkage map as well as the resulting QTL. The inclusion of Rrs_{Rhy017} moved three loci from the area with high marker density around the centromere to the distal part of the long arm of chromosome 3H. For Bmag0013 and GBM1037 this was already known as the correct position. Comparison with the consensus map of Stein et al. (2007) and Varshney et al. (2007) revealed that this was also the case for marker GBM1050. Rrs_{Rhy017} also had an effect on the QTL detected on chromosome 3H. The small second QTL observed before distally on the short arm of chromosome 3H was not detected this time with or without cofactors. The deduction from these findings was that the genetic map constructed with Rrs_{Rhy017} from approach A is probably the most precise one of the three presented in Fig-

ure 3.3-19. The small QTL on the short arm of chromosome 3H was potentially an artifact caused by the incorrect marker order.

Assuming that *Rrs*$_{Rhy017}$ is an allele of *Rrs1*, the results from the QTL analysis with the data from the Rhy174 experiment need to be considered. Rhy174 is one of the isolates used by Hofmann et al. (2013) to evaluate the efficacy of resistance donors SBCC145 and SBCC154. Both accessions contain only one resistance gene, *Rrs1*. And both accessions displayed good resistance against this isolate. *Rrs*$_{Rhy017}$ on the other hand shows almost no efficacy against this isolate. The implication is that while *Rrs*$_{Rhy017}$ very likely is an allele of *Rrs1*, it appears to be a different allele than the one present in SBCC145 and SBCC154. It is therefore suggested that the resistance gene present in the centromeric region of chromosome 3H of accession CIho3515 is denominated *Rrs1*$_{CIho3515}$, until the nature of the *Rrs1* locus has been fully clarified and an agreed nomenclature for the various alleles and/or loci has been agreed on.

3.4.3 Chromosome 6HS locus

As chromosome 6HS had already been identified as the carrier of the second resistance source of CIho3515, the aim was to construct genetic map for chromosome 6H of population DH33349 with a high marker density. The more specific region of interest was defined based on the results of the QTL analysis in DH761 as the region distally of SSR marker Bmag0500. In DH33349 20 marker were mapped into this region covering a genetic distance of 23.2 cM.

The QTL analysis based on this map and the data from the greenhouse experiments returned two QTL on chromosome 6H, one for isolate Rhy174 and one for isolate Sachs147-1. After adding cofactor scsnp07305 to the analysis a third QTL was detected for isolate LfL07. The position of all those QTL corresponded with the QTL detected in DH761. The resistance appears to be located a few cM proximally of the end of chromosome 6HS. Including a cofactor in the analysis had no influence on the location of the QTL.

Just like the locus on chromosome 3H, *Rrs1*$_{CIho3515}$, displayed no efficacy at all against isolate Rhy174 without cofactors, and only minimal efficacy after including cofactors in the analysis, an attempt was made to map the resistance locus on chromosome 6H as

a phenotypic marker using the data from this experiment. *Rrs*$_{Rhy174}$ mapped on the end of the short arm of chromosome 6HS using both approaches describend in chapter 3.3.2.3. *Rrs*$_{Rhy174}$ mapped in the same position as STS marker 2048_2 had mapped in population DH761. The influence on the marker order in the genetic map was minimal for both approach A and approach B. It was limited to a few small inversions only. The most notable difference between approaches was the distance between *Rrs*$_{Rhy174}$ and the next proximal marker, 4.9 cM for approach A and 10 cM for approach B. The influence of this phenotypic marker became apparent when conducting another QTL analysis. The original QTL was still present in the same position as before, but a second peak appeared in the position of *Rrs*$_{Rhy174}$. LOD scores decreased significantly between the two QTL. This effect was enhanced by including marker scsnp07305 as cofactor in the analysis. LOD scores between both QTL decreased almost to zero, and while the original more proximal QTL increased slightly, a significant decrease of the new distal QTL could be observed. Similar observations were made using the genetic maps derived from approach A as well as from approach B.

A possible explanation for the observed effects of *Rrs*$_{Rhy174}$ on the QTL analysis could be that, while the phenotypic marker is indeed strongly linked to the observed resistance, its position on the linkage group is not correct. *Rrs1*$_{Clho3515}$ has a small but nevertheless measurable effect in experiment as well. The effect could be strong enough to influence the disease scores which were used to map *Rrs*$_{Rhy174}$. The result could be an incorrect position of the marker as already suggested. As the linkage between the marker and the observed resistance is nevertheless very strong a QTL would be detected at its position. The same is applicable for the actual position of the resistance, the overall result being two QTL for the same resistance locus.

It is therefore very likely that the distal QTL is an artifact caused by the wrong position of the strongly linked marker *Rrs*$_{Rhy174}$, and that the true position of the resistance locus is indicated by the proximal QTL which was detected in DH761 already.

The short arm of chromosome 6HS is the putative position of scald resistance gene *Rrs13* (Abbott et al. 1995). A number of QTL for scald resistance have also been reported on this chromosome arm (Jensen et al. 2002, Cheong et al. 2006, Shtaya et al. 2006, Wagner et al. 2008). Comparison of all these results with the findings of the present work proves difficult as different genotypes, population sizes and markers or even marker systems were used in each case. In addition missing marker information potentially introduced a certain level of inaccuracy in each case (Close et al. 2009).

A direct comparison between maps from Abbott et al. 1995 and the map presented in this work for example is not possible as no common markers are present in both maps. A high density map by Rostoks et al. (2005, supplementary material) contains all markers used by Abbott et al. 1995 as well as This map was used as a cross walk between the map from Abbott et al. 1995 and the map from the present work (figure 3.4-1). According to this alignment the QTL detected in DH33349 is located considerably further distal on the chromosome arm than *Rrs13*.

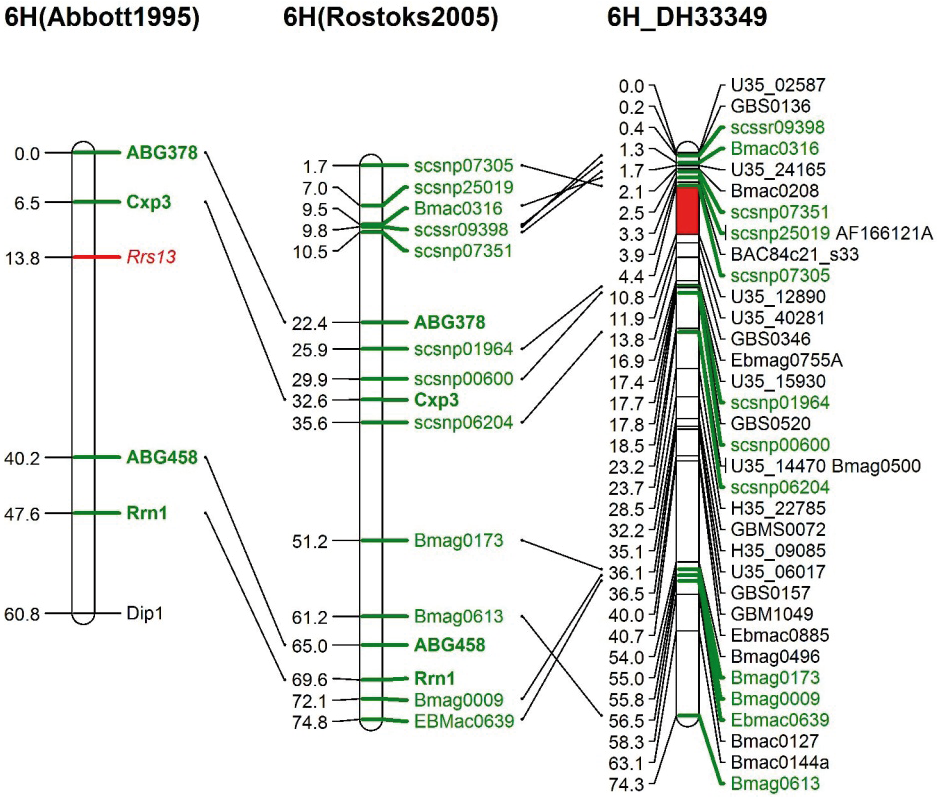

Figure 3.4-1: Alignment of genetic maps for chromosome 6H. Left: map by Abbott et al. (1995). Center: Map by Rostoks et al. (2005, supplementary material). Only markers present in either of the other two maps are displayed. Right: map created with population DH33349 in the present work.

Cheong et al. (2006) added two additional markers into the map from Abbott et al. 1995, SSR markers Bmag0500 and Bmag0173. This makes a direct comparison with the map from population possible (figure 3.4-2). The alignment of both maps confirms that the QTL detected in DH33349 is indeed located distally of the putative *Rrs13* location. It is therefore very likely a different locus from the *Rrs13* gene found by Abbott et al. (1995).

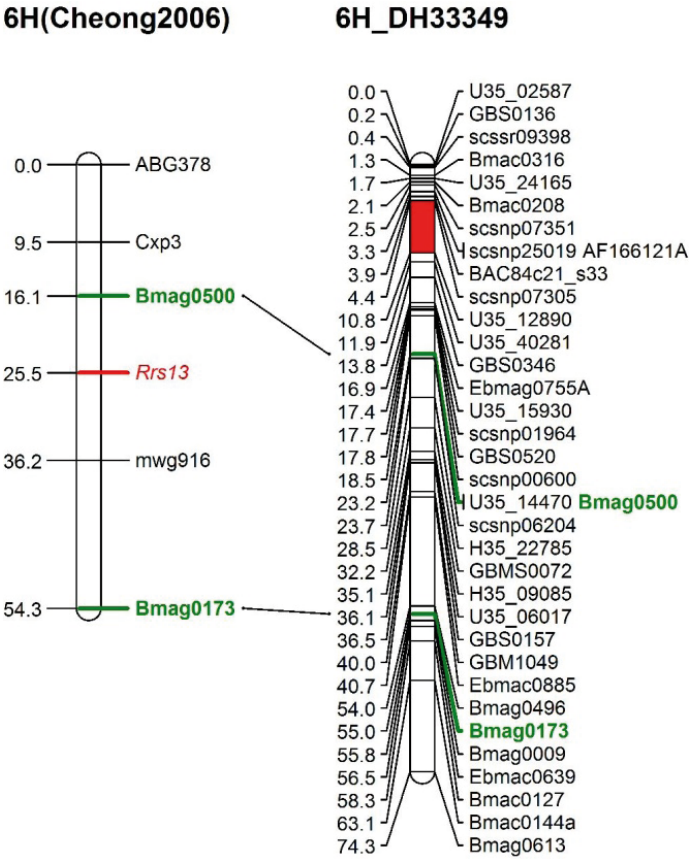

Figure 3.4-2: Alignment of genetic maps for chromosome 6H. Left: map by Cheong et al. (2006). Right: map created with population DH33349 in the present work.

The QTL found by Cheong et al. (2006) in three different cultivars all appear to be located in the same region of chromosome 6HS. The QTL from cv. O'Connor is located distally of SSR marker Bmag0500 and consequently *Rrs13* as well. It is therefore pos-

sible that the QTL found by Cheong et al. (2006) are identical or allelic to the QTL found in the present work.

Jensen et al. (2002) reported a QTL for scald resistance on chromosome 6HS coming from cv. Alexis. Alexis has been the susceptible parent of population DH33349, and has demonstrated no resistance at all in any of the experiments. It is therefore considered as highly unlikely that the QTL from Jensen et al. (2002) and the QTL from the present work are connected.

A comparison with the findings reported by Shtaya et al. (2006) is more difficult as no common marker exists in the maps. But the QTL identified by this group is located distally on chromosome 6HS, so it cannot be ruled out that the QTLs from Shtaya et al. (2006) and from this work are identical or allelic. The same rationale can be applied to the QTL located on chromosome 6HS by Wagner et al. (2008).

Most scald resistance genes that have been mapped are genes conferring seedling resistance while adult plant resistances (APR) have mostly been reported as QTL only (Cheong et al. 2006). In this work both types of resistance have been evaluated for the same locus. The resistance locus on chromosome 6HS of CIho3515 conveys seedling resistance to various isolates. The experiment with isolate Rhy174 demonstrated that this locus alone can convey medium to full resistance against scald. Marker assisted selection supports these results further. DH lines carrying only the resistance on chromosome 6HS (figs 3.3-31 and 3.3-32, genotype a/b) showed high levels of resistance in the experiment with isolate Sachs147-1, and medium levels of resistance in the experiment with isolate LfL07. The locus was also effective in the field, contributing to the observed resistance with 12%. MAS demonstrated that the locus can convey a high to medium field resistance against scald as well (figure 3.3-34).

Considering the broad efficacy of the locus and the high level of resistance conferred, the resistance locus on chromosome 6HS of CIho3515 should be considered a major resistance gene. The last new *Rrs* gene reported was the *Rrs16* locus which was introgressed from *Hordeum bulbosum* by Pickering et al. (2006). Zhan et al. (2008) proposed that the denomination *Rrs17* should be assigned to $Rrs15_{CIho8288}$ for the purpose of clarity.

The suggested designation for the resistance locus identified on chromosome 6HS in this work therefore is *Rrs18*.

3.4.4 Conclusions for practical barley breeding

Leaf scald is still a threat for barley production today. Several groups around the globe are working on the scald-barley-pathosystem to understand the interaction and find resources for resistance breeding (G. Schweizer, pers. comm.). Advisory services are monitoring the disease to support the farmers in their disease management, e.g. the Bavarian Crop State Research Center for Agriculture (LfL, http://www.lfl.bayern.de/ips/warndienst/030924/).

The threshold for making pesticide applications necessary is specified as 25-50% infected plants, depending on the region (agridea 2014, LfL 2005). The marker assisted selection reported in figure 3.3-34 reveals that each of the loci alone could be a valuable asset for keeping the level of infection below that threshold, assuming that rating scores 1 and 2 would be considered as acceptable.

Selecting for *Rrs1*$_{CIho3515}$ only with marker 11_0205 (figure 3.3-34 left) returns 157 out of 230 DH lines. 129 of these lines were rated with a disease score of 1 or 2. A population selected with 11_0205 only would therefore remain below the 25% threshold.

Marker scsnp07305, used to select for *Rrs18*, returns 92 of the 230 DH lines. This subpopulation contains 77 lines rated with 1 or two. Again the population remains below the 25% threshold.

A subpopulation of DH33349 selected with both markers would contain 56 DH lines, 54 of which were rated with 1 or 2. Selecting lines containing either or both loci would create a subpopulation of 193 individuals. The average disease score for these lines containing either or both loci is 1.55 (tab 3.3-18), while DH lines containing neither of the loci averaged at 3.43.

The overall conclusion is, that under field conditions as found in Bavaria, Germany, either of the resistance loci found in CIho3515 is sufficient to control leaf scald, and the markers used to select for these loci are suitable for MAS in breeding programs.

4 Summary

Leaf scald, caused by the fungal pathogen *Rhynchosporium commune*, is still one of the major diseases in barley growing regions around the globe. The main control strategy today is the application of pesticides. A more sustainable alternative is the use of resistant cultivars. This requires the successful integration of known resistance genes into the elite barley gene pool, as well as the identification of new resistance genes to ensure sufficient variability for resistance management. A prerequisite for any breeding program are tightly linked markers for successful selection of resistant progeny and to avoid linkage drag of undesirable traits.

Populations derived from three barley accessions of Spanish origin (SBCC145, SBCC154, CIho3515) were assessed for scald resistance in the greenhouse and field. Genetic maps were constructed to locate the resistance loci and identify markers tightly linked to these loci.

SBCC145 and SBCC154 were selected from the Spanish Barley Core Collection for their outstanding resistance against several isolates of *R. commune*. Genetic markers HVM0027 and STSagtc17 showed strong linkage with the observed resistance, giving reason to the assumption that scald resistance gene *Rrs1* might be present in both accessions. Subsequently both populations were genotype with the Illumina GoldenGate 1,536 SNP Assay and a QTL analysis was conducted. The analysis revealed a major QTL on the long arm of chromosome 3H very close to the centromere in both populations, confirming the *Rrs1* locus. A panel of differential isolates indicated that the allele present in both SBCC145 and SBCC154 is $Rrs1_{Rh4}$. $Rrs1_{Rh4}$ was mapped as a binary trait and included in a consensus map of both populations. Flanking markers are 11_0010 proximally and 11_0823 distally with 1.2 and 0.9 cM distance respectively.

CIho3515 has been well known for its reported consistently excellent performance in resistance tests elsewhere as well as in experiments conducted at the LfL. Many hypotheses had been postulated as to the resistance loci present in this accession. However an attempt to positively identify and map these loci has never been made. Assessment with five different *R. commune* isolates revealed two independent major resistance loci. SSR marker HVM0027 again showed strong linkage with the observed resistance, and a QTL analysis confirmed the presence of an *Rrs1* allele in CIho3515. The differential reaction to *R. commune* isolate Rhy174 revealed that this allele of *Rrs1* is different from the one present in SBCC145 and SBCC154. To avoid any confusion the designation $Rrs1_{CIho3515}$ was selected.

The second resistance locus present in CIho3515 was located on chromosome arm 6HS. The QTL for this resistance gene were located towards the distal end of the chromosome arm, refuting the initial assumption that CIho3515 might be carrying an allele of *Rrs13*. So far only QTL had been reported in this area. The apparently new resistance gene was therefore denominated *Rrs18*.

The population derived from CIho3515 was assessed in the field as well. Subsequent MAS revealed that both loci are sufficiently effective in the field to keep the infection level below the threshold for pesticide application. The MAS also confirmed that 11_0205 and scsnp07305 are suitable markers for $Rrs1_{CIho3515}$ and *Rrs18*, respectively.

In total, two different alleles of the *Rrs1* resistance gene, $Rrs1_{Rh4}$ and R$rs1_{CIho3515}$, as well as a new scald resistance locus, *Rrs18*, have been identified in this work. In addition markers are presented making these genes accessible for practical breeding. From a scientific point of view this work presents sound basis to further investigate the complex nature of the *Rrs1* locus, and to pinpoint the location of the newly identified locus *Rrs18* in barley.

5 Zusammenfassung

Die Blattfleckenkrankheit, verursacht durch das Pilzpathogen *Rhynchosporium commune*, ist weltweit eine der bedeutendsten Krankheiten im Gerstenanbau. Die heutzutage vorherrschende Bekämpfungsstrategie ist der Einsatz von Pestiziden. Eine nachhaltige Alternative stellt der Anbau resistenter Sorten dar. Das erfordert die erfolgreiche Integration von bekannten Resistenzgenen in den Elite-Genpool, sowie die Identifikation neuer Resistenzquellen, um eine ausreichende Variabilität zum Zwecke des Resistenzmanagements zu gewährleisten. Eine Voraussetzung für solche Zuchtprogramme sind eng gekoppelte Marker, um eine sichere Selektion der resistenten Nachkommen zu gewährleisten und zugleich unerwünschte Eigenschaften leicht eliminieren zu können.

Populationen basierend auf drei Gerstenakzessionen spanischer Herkunft (SBCC145, SBCC154, CIho3515) wurden im Gewächshaus und im Feld hinsichtlich ihrer *Rhynchosporium*-Resistenz geprüft. Genetische Karten wurden erstellt um die Resistenzquellen zu lokalisieren und eng gekoppelte Marker zu identifizieren.

Die Akzessionen SBCC145 und SBCC154 stammen aus der Spanish Barley Core Collection und wurden wegen ihrer herausragenden Resistenz gegenüber verschiedenen *R. commune*-Isolaten ausgewählt. Die genetischen Marker HVM0027 und STSagtc17 zeigten eine starke Kopplung mit der beobachteten Resistenz, und gaben somit Anlass zu der Annahme, dass das Resistenzgen *Rrs1* in beiden Akzessionen präsent sein könnte. Die Kartierung der Populationen mit dem Illumina GoldenGate 1,536 SNP Assay und eine nachfolgende QTL-Analyse ergaben einen bedeutenden QTL auf dem langen Arm von Chromosom 3H in der Nähe des Centromers, womit die Präsenz des *Rrs1*-Genlocus bestätigt wurde. Eine Auswahl differenzierender Isolate zeigte, dass es sich bei dem *Rrs1*-Allel in beiden Akzessionen um das $Rrs1_{Rh4}$-Allel handelt. $Rrs1_{Rh4}$ wurde als binäres Merkmal kartiert und in eine Konsensuskarte beider Populationen integriert. Flankierende Marker sind 11_0010 proximal mit 1,2 cM Abstand, und 11_0823 distal mit 0,9 cM Abstand.

CIho3515 ist aus der Literatur sowie aus Experimenten an der LfL für seine konsistent hohe Resistenz gegenüber *R. commune* bekannt, Die vermutlich auf mehreren Resistenzloci beruht. Diese Loci wurden bislang jedoch noch nicht eindeutig identifiziert und kartiert. Eine Resistenzprüfung mit fünf verschiedenen *R. commune*-Isolaten ergab zwei voneinander unabhängige voll wirksame Resistenzgene. Der Marker HVM0027 zeigte auch in dieser Population eine enge Kopplung mit der beobachteten Resistenz, und eine QTL-Analyse bestätigte das Vorliegen eines *Rrs1*-Allels in CIho3515. Die unterschiedliche Reaktion gegenüber *R. commune*-Isolat Rhy174 zeigte, dass es sich bei diesem Rrs1-Allel nicht um dasselbe Allel wie in SBCC145 und SBCC154 handelt; um für Klarheit zu sorgen, wurde für das „neue" Allel die Bezeichnung $Rrs1_{CIho3515}$ gewählt.

Das zweite Resistenzgen von cIho3515 wurde auf Chromosomenarm 6HS lokalisiert. QTL für dieses Gen wurden am distalen Ende des Chromosomenarmes gefunden. Somit wurde die ursprüngliche Annahme, dass es sich um eine mit *Rrs13* allelische Resistenz handeln könnte, widerlegt. In der betreffenden Region wurde bislang nur über QTL berichtet. Das offenbar neue Resistenzgen wurde daher mit der Bezeichnung *Rrs18* versehen.

Die auf CIho3515 basierende Population wurde außerdem im Feld geprüft. Eine darauf basierende markergestützte Selektion zeigte, dass beide Resistenzgene ausreichend effektiv sind, um den Befall mit der Blattfleckenkrankheit unter der Bekämpfungsschwelle zu halten. Dieser Ansatz bestätigte weiterhin die Eignung der gewählten Marker 11_0205 und scsnp07305 für die Selektion auf $Rrs1_{CIho3515}$ und *Rrs18*.

Zusammenfassend wurden in der vorliegenden Arbeit zwei verschiedene Allele der Rrs1-Gens, $Rrs1_{Rh4}$ und R$rs1_{CIho3515}$, sowie ein neues Resistenzgen, *Rrs18*, gegen *R. commune* gefunden. Zusätzlich wurden Marker identifiziert, die diese Resistenzquellen für Zuchtprogramme verfügbar machen. Vom wissenschaftlichen Standpunkt aus stellt diese Arbeit eine sehr gute Basis dar, um die komplexe Natur des *Rrs1*-Genkomplexes weiter zu analysieren, und die Position des neu gefundenen Resistenzgenes *Rrs18* im Genom weiter einzugrenzen.

6 References

Abbott DC, Burdon JJ, Jarosz AM, Brown AHD, Muller WJ, Read BJ (1991) The relationship between seedling infection types and field reactions in Clipper barley backcross lines. Aust J Agric Res 42:801-809

Abbott DC, Brown AHD, Burdon JJ (1992) Genes for scald resistance from wild barley (*Hordeum vulgare* ssp *spontaneum*) and their linkage to isozyme markers. Euphytica 61:225-231

Abbott DC, Lagudah ES, Brown AHD (1995) Identification of RFLPs Flanking a Scald Resistance Gene on Barley Chromosome 6. J Heredity 86(2):152-154

Aghnoum R, Marcel TC, Johrde A, Pecchioni N, Schweizer P, Niks RE (2010) Basal host resistance of barley to powdery mildew: connecting quantitative trait loci and candidate genes. MPMI 23:91–102

agridea (2014) Datenblätter Ackerbau: Bekämpfungsschwellen. www.agridea.ch/Fachbereiche/Pflanzenbau/Ackerbau. Accessed 25 September 2014

Ali SM (1974) Factors Infuencing Infection, Colonization and Symptom Expression in Barley by *Rhynchosporium secalis*. Aust J Agric Res 25:9-20

Ali SM, Mayfield AH, Clare BG (1976) Pathogenicity of 203 isolates *of Rhynchosporium secalis* on 21 barley cultivars. Physiol Plant Pathol 9:135-143

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new Res 25:3389–3402

Avrova A, Knogge W (2012) *Rhynchosporium commune*: a persistent threat to barley cultivation. Mol Plant Pathol 13:986–997

Ayres G, Owen H (1971) Resistance of barley varieties to establishment of subcuticular mycelia by *Rhynchosporium secalis*. Trans Br Mycol Soc 57:233–240

Baba T, Tanno K, Furusho M, Komatsuda T (2011) Allelic variation at the EF-G locus among northern Moroccan six-rowed barleys. Plant Genetic Resour: Charact Util 9:240–242

Badr A, Müller K, Schäfer-Pregl R, El Rabey H, Effgen S, Ibrahim HH, Pozzi, Rhode W, Salamini F (2002) On the Origin and Domestication History of Barley (*Hordeum vulgare*). Mol Biol Evol 17(4):499–510

Barua UM, Chalmers KJ, Hackett CA, Thomas WTB, Powell W, Waugh R (1993) Identification of RAPD markers linked to a *Rhynchosporium secalis* resistance locus in barley using near-isogenic lines and bulked segregant analysis. Heredity 71:177-184

Behn A, Hartl L, Schweizer G, Wenzel G, Baumer M (2004) QTL mapping for resistance against non-parasitic leaf spots in a spring barley doubled haploid population. Theor Appl Genet 108:1229–1235

Bjørnstad Å, Patil V, Tekauz A, Marøy AG, Skinnes H, Jensen A, Magnus H, MacKey J (2002) Resistance to scald (*Rhynchosporium secalis*) in barley (*Hordeum vulgare*) studied by near-isogenic lines. I. Markers and differential isolates. Phytopathol 92:710–720

BMEL Bundesministerium für Landwirtschaft und Ernährung (2014) Erntebericht 2014. www.bmel.de/SharedDocs/Pressemitteilungen/2014/195-SC-Erntebericht2014.html. Accessed 25 September 2014

Caldwell RM (1937) *Rhynchosporium* scald of barley, rye and other grasses. J Agric Res 55:175–198.

Casao MC, Igartua E, Karsai I, Lasa JM, Gracia MP, Casas AM (2011) Expression analysis of vernalization and day-length response genes in barley (*Hordeum vulgare* L.) indicates that *VRNH2* is a repressor of *PPDH2* (*HvFT3*) under long days. J Exp Bot 6:1939–1949

Cheong J, Williams K, Wallwork H (2006) The identification of QTLs for adult plant resistance to leaf scald in barley. Aust J Agric Res 57:961–965

Close TJ, Bhat PR, Lonardi S, Wu Y, Rostoks N, Ramsay L, Druka A, Stein N, Svensson J, Wanamaker S, Bozdag S, Roose M, Moscou M, Chao S, Varshney R, Szucs P, Sato K, Hayes P, Matthews D, Kleinhofs A, Muehlbauer G, DeYoung J, Marshall D, Madishetty K, Fenton R, Condamine P, Graner A, Waugh R (2009) Development and implementation of high-throughput SNP genotyping in barley. BMC Genomics 10:582

Collinge DB, Kragh KM, Mikkelsen JD, Nielsen KK, Rasmussen U, Vad K (1993) Plant chitinases. Plant J 3:31–40

Churchill GA, Doerge RW (1994) Empirical Threshold Values for Quantitative Trait Mapping. Genetics 138:963-971

Dyck PL, Schaller CW (1961) Inheritance of resistance in barley to several physiologic races of the scald fungus. Can J Genet Cytol 3:153–164

Frank A (1897). Über die Zerstörung durch einen neuen Gerste-Pilz. Wochenschrift Brauerei 14:518–520

Friedt W, Ordon F (2013) Barley Production and Breeding in Europe: Modern Cultivars Combine Disease Resistance, Malting Quality and High Yield. In: Advance in Barley Science - Proceedings of 11th International Barley Genetics Symposium, 2013: pp 389-400. ISBN: 978-94-007-4681-7 (Print) 978-94-007-4682-4 (Online)

Fukuyama T, Yamaji S, Nakamura H (1998) Differentiation of Virulence in *Rhynchosporium secalis* in the Hokuriku District and Sources of Resistance to the Pathogen. Breeding Science 48:23-28

Garvin DF, Brown AHD, Raman H, Read BJ (2000) Genetic mapping of the barley *Rrs14* scald resistance gene with RFLP, isozyme and seed storage protein markers. Plant Breed 119:193–196

Genger RK, Brown AHD, Knogge W, Nesbitt K, Burdon JJ (2003) Development of SCAR markers linked to a scald resistance gene derived from wild barley. Euphytica 134:149–159

Genger RK, Nesbitt K, Brown AHD, Abbott DC, Burdon JJ (2005) A novel barley scald resistance gene: genetic mapping of the *Rrs15* scald resistance gene derived from wild barley, *Hordeum vulgare* ssp. *spontaneum*. Plant Breeding 124:137-141

Goodwin SB, Allard RW, Webster RK (1990) A Nomenclature for *Rhynchosporium secalis* Pathotypes. Phytopathology 80(12):1330-1336

Graner A, Tekauz A (1996) RFLP mapping of a dominant gene conferring resistance to scald (*Rhynchosporium secalis*). Theor Appl Genet 93:421–425

Grønnerød S, Marøy AG, MacKey I, Tekauz A, Penner GA, Bjørnstad A (2002) Genetic analysis of resistance to barley scald (*Rhynchosporium secalis*) in the Ethiopian line 'Abyssinian' (CI668). Euphytica 126:235–250

Habgood MR, Hayes JD (1971) The inheritance of resistance to *Rhynchosporium secalis* in barley. Heredity 27:25–37

Hahn M, Jüngling S, Knogge W (1993) Cultivar-Specific Elicitation of Barley Defense Reactions by the Phytotoxic Peptide NIP1 from *Rhynchosporium secalis*. MPMI 6(6):745-754

Hanemann A, Schweizer GF, Cossu R, Wicker T, Röder MS (2009) Fine mapping, physical mapping and development of diagnostic markers for the *Rrs2* scald resistance gene in barley. Theor Appl Genet 119:1507–1522

Hartl L, Mohler V, Zeller FJ, Hsam SLK, Schweizer G (1999). Identification of AFLP markers closely linked to the powdery mildew resistance genes *Pm1c* and *Pm4a* in common wheat. Genome 42:322–329.

Heitefuss R, König K, Obst A, Reschke M (2000) Pflanzenkrankheiten und Schädlinge im Ackerbau (4. Auflage). DLG-Verlag, Frankfurt/Main.

Hillocks RJ (2012) Farming with fewer pesticides: EU pesticide review and resulting challenges for UK agriculture. Crop Prot 31:85–93

Hofmann K, Silvar C, Casas AM, Herz M, Büttner B, Garcia MP, Contraeras-Moreira B, Wallwork H, Igartua E, Schweizer G (2013) Fine mapping of the Rrs1 resistance locus against scald in two large populations derived from Spanish barley landraces. Theor Appl Genet 126:3091-3102

IBSC International Barley Genome Sequencing Consortium (2012) A physical, genetic and functional sequence assembly of the barley genome. Nature 491:711–716

Igartua E, Gracia MP, Lasa JM, Medina B, Molina-Cano JL, Montoya JL, Romagosa I (1998) The Spanish barley core collection. Genet Resour Crop Ev 45:475–481

Igartua E, Moralejo M, Casas AM, Torres L, Molina-Cano JL (2013) Whole-genome analysis with SNPs from BOPA1 shows clearly defined groupings of Western Mediterranean, Ethiopian, and Fertile Crescent barleys. Genet Resour Crop Ev 60:251–264

Jackson LF, Webster RK (1976) Race differentiation, distribution, and frequency of *Rhynchosporium secalis* in California. Phytopathol 66:719–725

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

Jensen J, Backes G, Skinnes H, Giese H (2002) Quantitative trait loci for scald resistance in barley localized by a non-interval mapping procedure. Plant Breed 121:124–128

Jørgensen HJL, de Neergaard E, Smedegaard-Petersen V (1993) Histological examination of the interaction between *Rhynchosporium secalis* and susceptible and resistant cultivars of barley. Physiol Mol Plant Pathol 42:345-358

Koressaar T, Remm M (2007) Enhancements and modifications of primer design program Primer3 Bioinformatics 23(10):1289-91

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Langridge P, Karakousis A, Kretschmer J, Manning S, Chalmers K (1996) Molecular relationship between barley varieties. http://wheat.pw.usda.gov/ggpages/DEM/Polymorphism/Waite/Barley_dend.gif. Accessed 26 Feb 2013

Lasa JM (2008) Spanish barley core collection. Monografias INIA: Serie Agricola, n25. ISBN 978-84-7498-526-9

LfL Bayerische Landesanstalt für Landwirtschaft (2005) Gerstenkrankheiten – Diagnose- und Entscheidungshilfen zum Gerstenmodell Bayern. LfL-Informationen, 4. geänderte Auflage, November 2005

Li HB, Zhou MX (2011) Quantitative trait loci controlling barley powdery mildew and scald resistances in two different barley doubled haploid populations. Mol Breed 27:479–490

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

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

Looseley ME, Newton AC, Atkins SD, Fitt BDL, Fraije B, Thomas WTB, Keith R, Lynott J, Harrap D (2012) Genetic basis of control of *Rhynchosporium secalis* infection and symptom expression in barley. Euphytica 184:47–56

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

Mayer KFX, Martis M, Hedley PE, Šimková H, Liu H, Morris JA, Steuernagel B, Taudien S, Roessner S, Gundlach H, Kubaláková M, Suchánková P, Murat F, Felder M, Nussbaumer T, Graner A, Salse J, Endo T, Sakai H, Tanaka T, Itoh T, Sato K, Platzer M, Matsumoto T, Scholz U, Dolezél J, Waugh R, Stein N (2011) Unlocking the barley genome by chromosomal and comparative genomics. Plant Cell 23:1249–1263

Muñoz-Amatriaín M, Moscou MJ, Bhat PR, Svensson JT, Bartoš J, Suchánková P, Šimková H, Endo TR, Fenton RD, Lonardi S, Castillo AM, Chao S, Cistué L, Cuesta-Marcos A, Forrest KL, Hayden MJ, Hayes PM, Horsley RD, Makoto K, Moody D, Sato K, Vallés MP, Wulff BBH, Muehlbauer GJ, Doležel J, Close TJ (2011) An improved

consensus linkage map of barley based on flow-sorted chromosomes and single nucleotide polymorphism markers. Plant Genome 4:238–249

Oudemans CAJA (1897) Observations mycologiques. Koninklijke Nederlandse Akaddemie van Wetenschapen Amsterdam 6: 86–92.

Patil V, Bjørnstad A, Mackey J (2003) Molecular mapping of a new gene R*rs4*$_{CI11549}$ for resistance to barley scald (*Rhynchosporium secalis*). Mol Breed 12:169–183

Paulitz TC, Steffenson BJ (2011) Biotic stress in barley: disease problems and solutions. In: Ullrich SE (ed) Barley: production, improvement and uses. Blackwell Publishing Ltd, London, pp 307–354

Penner GA, Tekauz A, Reimer E, Scoles GJ, Rossnagel BG, Eckstein PE, Legge WG, Burnett PA, Ferguson T, Helm JF (1996) The genetic basis of scald resistance in western Canadian barley cultivars. Euphytica 92:367–374

Phillips D, Nibau C, Ramsay L, Waugh R, Jenkins G (2010) Development of a molecular cytogenetic recombination assay for barley. Cytogenet Genome Res 129:154–161

Pickering R, Ruge-Wehling B, Johnston PA, Schweizer G, Ackermann P, Wehling P (2006) The transfer of a gene conferring resistance to scald (*Rhynchosporium secalis*) from *Hordeum bulbosum* into *H. vulgare* chromosome 4HS. Plant Breed 125:576–579

Ponce-Molina LJ, Casas AM, Gracia MP, Silvar C, Mansour E, Thomas WBT, Schweizer G, Herz M, Igartua E (2012) Quantitative trait loci and candidate loci for heading date in a large population of a wide barley cross. Crop Sci 52:2469–2480

Ramsay L, Macaulay M, degli Ivanissevich S, 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

Read BJ, Macdonald AF (1991) *Hordeum vulgare* (barley) cv. Yerong. Aust J Exp Agric 31:866

Robbertse B, Lennox CL, van Jaarsveld AB, Crous PW, van der Rijst M (2000) Pathogenicity of the *Rhynchosporium secalis* population in the Western Cape province of South Africa. Euphytica 115:75-82

Rostoks N, Mudie S, Cardle L, Russell J, Ramsay L, Booth A, Svensson J, Wanamaker S, Walia H, Rodriguez E, Hedley P, Liu H, Morris J, Close T, Marshall D, 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

Salamati S, Tronsmo AM (1997) Pathogenicity of the *Rhynchosporium secalis* population in the Western Cape province of South Africa. Plant Pathol 46:416-424

Sayed H, Kayyal H, Ramsay L, Ceccarelli S, Baum M (2002) Segregation distortion in doubled haploid lines of barley (*Hordeum vulgare* L.) detected by simple sequence repeat (SSR). Euphytica 225:265–272

Schein RD (1958) Pathogenic specialization in *Rhynchosporium secalis*. Phytopathology 48:477–480

Schweizer GF, Baumer M, Daniel G, Rugel H, Röder MS (1995) RFLP markers linked to scald (*Rhynchosporium secalis*) resistance gene *Rh2* in barley. Theor Appl Genet 90:920–924

Schweizer G, Herz M, Mikolajewski S, Brenner M, Hartl L, Baumer M (2004) Genetic mapping of a novel scald resistance gene $Rrs15_{CI8288}$ in barley. In: Proceedings of 9th Int Barley Genet Symposium, Brno, Czech Republic, 20–26 June 2004, pp 258–265

Shipton WA, Boyd WJR, Ali SM (1974) Scald of Barley. Rev Plant Pathol 53:839–861

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

Siersleben S, Penselin D, Wenzel C, Albert S, Knogge W (2014) *PFP1*, a gene encoding an Epc-N domaincontaining protein, is essential for pathogenicity of the barley pathogen *Rhynchosporium commune*. Eucaryotic Cell 13(8):1026-1035

Silvar C, Casas AM, Kopahnke D, Habekuß A, Schweizer G, Gracia MP, Lasa JM, Ciudad FJ, Molina-Cano JL, Igartua E, Ordon F (2010) Screening the Spanish barley core collection for disease resistance. Plant Breed 129:45–52

Silvar C, Casas AM, Igartua E, Ponce-Molina LJ, Gracia MP, Schweizer G, Herz M, Flath K, Waugh R, Kopahnke D, Ordon F (2011) Resistance to powdery mildew in Spanish barley landraces is controlled by different sets of quantitative trait loci. Theor Appl Genet 123:1019–1028

Starling TM, Roane CW, Chi KR (1971) Inheritance of reaction to *Rhynchosporium secalis* in winter barley cultivars. In: Proceedings of 2nd Int Barley Genetics Symposium, Pullman, WA, pp 513–519

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 114:823–839

Steiner-Lange S, Fischer A, Boettcher A, Rouhara I, Liedgens H, Schmelzer E, Knogge W (2003) Differential defense reactions in leaf tissues of barley in response to infection by *Rhynchosporium secalis* and to treatment with a fungal avirulence gene product. Mol Plant-Microbe Interactions 16:893–902

Tekauz A (1991) Pathogenic variation in *Rhynchosporium secalis* on barley Canada. Can J Plant Pathol 13:298-304

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

Thiel T, Kota R, Grosse, I Stein N, Graner A (2004) SNP2CAPS: a SNP and INDEL analysis tool for CAPS marker development. Nucleic Acids Res 32(1):e5

Thirugnanasambandam A, Wright KM, Atkins SD, Whisson SC, Newton AC (2011) Infection of *Rrs1* barley by an incompatible race of the fungus *Rhynchosporium secalis* expressing the green fluorescent protein. Plant Pathol 60:513–521

Thomas WTB, Powell W, Waugh R, Chalmers KJ, Barua UM, Jack P, Lea V, Forster BP, Swanston JS, Ellis RP, Hanson PR, Lance RCM (1995) Detection of quantitative trait loci for agronomic, yield, grain and disease characters in spring barley (*Hordeum vulgare* L.). Theor Appl Genet 91:1037–1047

Untergrasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG (2012) Primer3 - new capabilities and interfaces. Nucleic Acids Research 40(15):e115

Utz, H.F., and A.E. Melchinger, 1996: PLABQTL: A program for composite interval mapping of QTL. J. Agric. Genomics 2(1). Advance in Barley Sciences 2013, pp 389-400

van Ooijen JW (2004) MapQTL® 5, Software for the mapping of quantitative trait loci in experimental populations. Kyazma BV, Wageningen

van Ooijen JW (2006) JoinMap 4, software for the calculation of genetics linkage maps in experimental populations. Kyazma BV, Wageningen

van't Slot KAE, van den Burg HA, Kloks CPAM, Hilbers CW, Knogge W, Papavoine CHM (2003) Solution Structure of the Plant Disease Resistance-triggering Protein NIP1 from the Fungus *Rhynchosporium secalis* Shows a Novel β-Sheet Fold. J Biol Chem 278(46):45730-45736

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

Varshney RK, Marcel TC, Ramsay L, Russell J, Röder MS, 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

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

Voorrips RE (2002) MapChart: Software for the graphical presentation of linkage maps and QTLs. J Heredity 93(1):77-78.

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

VSN International (2011) Genstat for Windows 14th edn. VSN International, Hemel Hempstead, UK http://www.vsni.co.uk/software/genstat?ref=genstat.co.uk. Accessed 03 Oct 2011

Wagner C, Schweizer G, Krämer M, Dehmer-Badani AG, Ordon F, Friedt W (2008) The complex quantitative barley—*Rhynchosporium secalis* interaction: newly identified QTL may represent already known resistance genes. Theor Appl Genet 118:113–122

Wallwork H, Grcic M (2011) The use of differential isolates of *Rhynchosporium secalis* to identify resistance to leaf scald in barley. Aust Plant Pathol 40:490–496

Wells SA, Skoropad WP (1963) Inheritance of reaction to *Rhynchosporium secalis* in barley. Can J Plant Sci 43:184-187

Wenzel C (2014) Identifizierung und Charakterisierung von putativen Virulenzfaktoren des Gerstenpathogens *Rhynchosporium commune*. Dissertation, Naturwissenschaftliche Fakultät I, Universität Halle-Wittenberg

Wevelsiep L, Rüpping E, Knogge W (1993) Stimulation of Barley Plasmalemma Peptides from the Fungal Pathogen H^+-ATPase by Phytotoxic *Rhynchosporium secalis*. Plant Physiol 101:297-301

Williams KJ, Bogacki P, Scott L, Karakousis A, Wallwork H (2001) Mapping a gene for scald resistance in barley Line 'B87/14' and validation of microsatellite and RFLP markers for marker-assisted selection. Plant Breed 120:301–304

Xi K, Burnett PA, Tewari JP, Chen MH, Turkington TK, Helm JH (2000a) Histopatho-logical Study of Barley Cultivars Resistant and Susceptible to *Rhynchosporium secalis*. Phytopathology 90(1):94-102

Xi K, Xue AG, Burnett PA, Helm JH, Turkington TK (2000b) Quantitative resistance of barley cultivars to *Rhynchosporium secalis*. Can J Plant Pathol 22:221–227

Yun SJ, Gyenis L, Hayes PM, Matus I, Smith KB, Steffenson BJ, Muehlbauer GJ (2005) Quantitative trait loci for multiple disease resistance in wild barley. Crop Sci 45:2563–2572

Zaffarano PL, McDonald BA, Linde CC (2011) Two new species of *Rhynchosporium*. Mycologia 103:195–202

Zhan J, Fitt BDL, Pinnschmidt HO, Oxley SJP, Newton AC (2008) Resistance, epidemiology and sustainable management of *Rhynchosporium secalis* populations on barley. Plant Pathol 57:01–14

Zhan J, Yang L, Zhu W, Shang L, Newton AC (2012) Pathogen populations evolve to greater race complexity in agricultural systems— evidence from analysis of *Rhynchosporium secalis* virulence data. PLoS One 6:e38611

Zohary D, Hopf M (2000) Domestication of plants in the Old World. 3rd edition, Oxford University Press

Acknowledgements

I want to express my gratitude towards Prof. Wolfgang Friedt for supervising this work. His support and his advice ever since my time as an undergraduate student at the institute have been invaluable and highly influential for my professional life and career.

My gratitude also belongs to Günther Schweizer, Bayerische Landesanstalt für Landwirtschaft (LfL), Freising, for entrusting this project to me and supporting my work and my proposals with all available resources.

Special thanks go to Alfred Barth and Alexandra Jestadt. Alfred's indefatigable commitment in the lab and especially in the greenhouse made this project possible and ensured the consistently high quality of the greenhouse assay data. Alex has been a highly diligent support in the lab, managing, conducting and delegating the considerable amount of lab work efficiently and independently.

My appreciation also goes to Markus Herz, Rudi Cais and Christine Paternoster from the barley breeding group. Neither the field trials nor the greenhouse assays would have been possible without their support.

I would also like to express my thanks to Cristina Silvar, Ana Casas and Ernesto Igartua for the productive and instructive cooperation.

To Petra Hager and Stefan Seefelder I owe infinite gratitude for their loyalty, their moral support and their time and patience whenever it was needed.

I want to thank Tina Schwab for her work in the lab on population DH761, Manuela Diethelm, who supported me with the analysis of the first sequence data obtained from this project, Susanne Wüllner, who had her share of the substantial amount of lab work this project created, and Bianca Büttner for proof-reading the manuscript for this work.

Finally I want to express my heartfelt thanks to my family and friends, whose unconditional love, patience and unswerving belief and support kept me going.

This work was funded by the Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz (BMELV) via the Bundesanstalt für Landwirtschaft und Ernährung (BLE) through the project "*Rhynchosporium secalis*-resistance in barley" (Project 28-1-41.009-06).

Declaration

“I declare that I have completed this dissertation single-handedly without the unauthorized help of a second party and only with the assistance acknowledged therein. I have appropriately acknowledged and referenced all text passages that are derived literally from or are based on the content of published or unpublished work of others, and all information that relates to verbal communications. I have abided by the principles of good scientific conduct laid down in the charter of the Justus-Liebig-University of Giessen in carrying out the investigations described in the dissertation.”

Eidesstattliche Erklärung

„Hiermit erkläre ich: Die vorgelegte Dissertation habe ich selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsatze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.“

Gießen, 30. September 2014

Kerstin Hofmann