High Resolution Mapping of $Rph_{MBR1012}$ Conferring Resistance to *Puccinia hordei* in Barley (*Hordeum vulgare* L.)

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1 General introduction

1.1 Barley history and importance

Barley (*Hordeum vulgare* L ssp. *vulgare*) is a cereal crop that belongs to the family of grasses (*Poaceae*), which is of prime economic importance in agriculture and food industry. Due to the high ecological adaptability, barley is one of the most widely grown crops worldwide. Taxonomically, barley belongs to the order *Poales*, the family *Poaceae*, the tribe *Triticeae*, and the genus *Hordeum* (ITIS 2017). The genus *Hordeum* includes 32 different species and consists of diploid, tetraploid and hexaploid (Taketa et al. 2001) as well as annual and perennial species (Bothmer et al. 2003). The species *Hordeum vulgare* is classified into the subspecies *H. vulgare* ssp. *vulgare* (cultivated barley) and *H. vulgare* ssp. *spontaneum* (wild, barley).

Morphologically, *Hordeum* has been considered as a monophyletic plant group characterized by three one-flowered spikelets at each rachis node. The two lateral ones are pedicellate, rudimentary or sterile in two rowed barley, and the central one is sessile (rarely pedicellate). Barley itself consists of different types, i.e. two-rowed, six rowed, naked, hulled and hooded types. The chromosome number of *Hordeum* species is n = 7 at different ploidy levels which is diploid (2n = 2x = 14) for cultivated barley (*Hordeum vulgare* L. ssp. *vulgare*), which is usually a self-pollinated crop and its wild relative *Hordeum spontaneum* (C. Koch) while other wild species, i.e. *H. brachyantherum* ssp. *brachyantherum* (4x), *H. secalinum* (4x), *H. capense* (4x) and *H. brachyantherum* ssp. *brachyantherum* (6x) are tetraploid (2n = 4x = 28) or hexaploid (2n = 6x = 42). Most annuals in the genus *Hordeum* are inbreeding species whereas perennials are variable concerning pollination (von Bothmer and Jacobsen 1986, Komatsuda et al. 1999). Barley is highly adapted to a wide range of environmental conditions (Zohary and Hopf 2000) and is therefore grown from 70° N in Norway to 46° S in Chile. In terms of consumption. 55-60% of the produced barley is used for animal and livestock feeding. It is also used for malting (30-40%) and direct human consumption (5-10%) (Baik and Ullrich 2008, Blake et al. 2011).

The physiological and morphological variation in barley reflects a large genetic diversity that eases the environmental adaptation of barley (Graner et al. 2003). Barley has several attributes that makes it best suited as a model system, i.e. a diploid genome, easy cultivation under a wide range of environmental conditions and extensive genetic resources and inbreeding (Harwood 2019). Although barley has a large genome size, at approximately 5.3 Gbp, there are extensive genomic resources including a 9K and 50K iSelect SNP chip and a high-quality barley reference genome sequence (Comadran et al. 2012, Bayer et al. 2017, Mascher et al. 2017). In barley also a range of biotechnology tools including doubled haploid production, efficient transformation,

TILLING, and more recently efficient genome editing, is available (Salvi et al. 2014, Kumlehn 2016).

1.2 World production and uses

In terms of seed production, following wheat, maize, and rice, barley is the fourth most grown cereal worldwide, with an area harvested of 46.9 million ha and a production totalling 141.3 million tons in 2016 (FAOSTAT 2018). More than 61% of barley produced between the years 2010 to 2017 was from Europe (Figure 1). In Europe, the barley production decreased by around 13.65 million tonnes, from 65.654 million tonnes in 2008 to 51.998 million tonnes in 2011 (Eurostat 2018). From 2011 to 2015 the barley production increased by 9.904 million tonnes and reached up to 61.902 million tonnes (Figure 2).

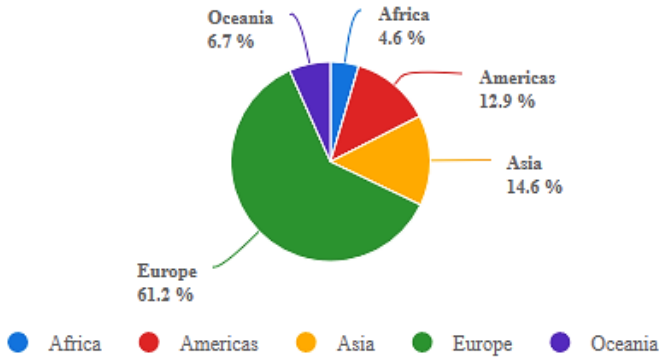

Figure 1. Production share of barley by region (average 2010-2017). Data sources: (http://www.fao.org/faostat/en/#data/QC/visualize).

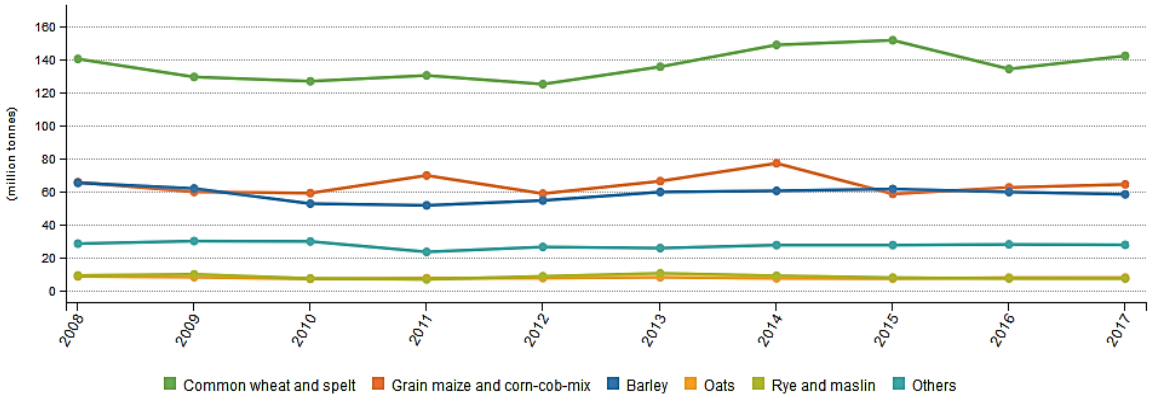

Figure 2. Production of main cereals, EU-28, 2008–2017 (https://ec.europa.eu/eurostat/statistics-explained/index.php/Agricultural_production_-_crops).

Regarding winter barley production in Germany, Bavaria (17%) is first followed by Lower Saxony and North Rhine-Westphalia, i.e. 15% and 14%, respectively (Figure 3).

Figure 3. Germany: Winter Barley, Percent of total winter barley area (60% of total barley area) Data sources: (https://www.usda.gov/oce/weather/pubs/Other/MWCACP/europe.htm).

1.3 Barley Leaf Rust and economic importance

In the late 1890s and early 1900s, rust diseases in cereal crops got more attention by plant pathologists. Three main rust diseases currently threaten barley production i.e. leaf rust (*Puccinia hordei*), stem rust (*Puccinia graminis* f. sp. *tritici*), and stripe rust (*Puccinia striiformis* sp. *hordei*) (Dracatos et al. 2019). Leaf rust is one of the most destructive and important diseases which affects barley worldwide (Park 2003). All three rust diseases affect malting quality through reductions in kernel plumpness, kernel weight, and germination, resulting in large economic losses (Roelfs 1978, Dill-Macky et al. 1990, Steffenson 1992, Qi et al. 1998). Barley leaf rust occurs to a great extent in both winter and spring barley production areas of the Eastern and Midwestern United States, North Africa, New Zealand, Europe, Australia, and parts of Asia (Mathre 1982). The pathogen is of particular importance in the regions where the crop matures late in the growing season (Park et al. 2015). The symptoms of barley leaf rust may range from small chlorotic flecks to large pustules containing spores. The leaf rust disease causes an increased plant transpiration and respiration, resulting in reduction of net photosynthesis rate and efficiency (Cotterill et al. 1992a, Das et al. 2007, Helfer 2014). Therefore, barley leaf rust affects plant and root growth and

lowers grain yield and seed quality (Mathre 1982, Wiese 1987). Yield losses are highest when leaf rust develops early in the season or when the crop is sown late (Melville et al. 1976, Mathre 1982). Reduction in kernel weight is reported as a major reason of yield losses due to leaf rust epidemics (Cooke 2006). An increase in the number of leaf rust races is the response to selection pressure exerted by resistance genes in barley cultivars (Mathre 1982).

Like other rust fungi, *P. hordei* is an obligate parasite that grows only on living tissue. *P. hordei* is a macrocyclic and heteroecious rust forming many cycles of urediniospores in one season (Schafer et al. 1984, Clifford 1985) and it requires more than one host to complete its life cycle. Macrocyclic rust in general has two stages in its life and an infection cycle that occurs in two hosts, the main host and the alternative host. During inter-cropping periods, the fungus survives as urediniospores or dormant mycelium on wild, volunteer, or over-wintering barley crops (Gair et al. 1987, Murray et al. 1998). Urediniospores are distributed by wind (Mathre 1982, Reinhold and Sharp. 1982, Wiese 1987). After contact of urediniospores with a receptive host, they germinate and penetrate to the leaf epidermis. Host penetration is completed within six to eight hours when free moisture and temperatures between 15°C and 25°C are available. Secondary urediniospores are produced in seven to ten days (Mathre 1982, Wiese 1987, Murray et al. 1998). After infection, rust pustules are formed predominantly on the upper side of the leaf sheaths (Zillinsky 1983, Wiese 1987). The tilletia stage is initiated 14 days after infection on barley towards the end of the growing season. The alternate host of *P. hordei* is the arable weed (a weed that is found in tilled cropland) *Ornithogalum umbellatum L.*, which is of no significance for the occurrence of leaf rust in central Europe (Schafer et al. 1984, Clifford 1985). The alternate host is infected when the teliospores germinate in the presence of free moisture. Basidiospores are produced that are capable of being carried a short distance (a few meters) to infect the alternate hosts (Schafer et al. 1984). Approximately seven to ten days following infection, pycnia and aecia are appeared. The aeciospores once landed on host leaves produce germ tubes and invade through stomata, resulting in the formation of pustules called uredosori that break through the epidermis and form urediniospores (Schafer et al. 1984, Clifford 1985). The alternative host can thus be a source of aeciospores providing an another way of spreading (Murray et al. 1998) (Figure 4).

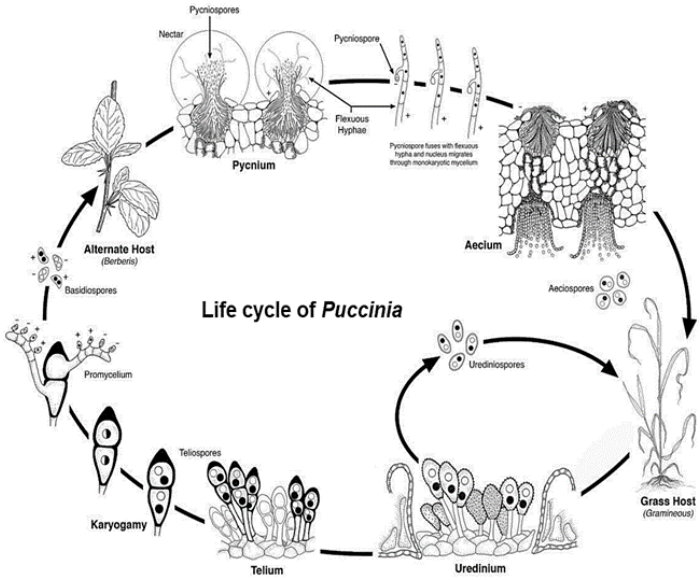

Figure 4. Complete life cycle of fungus *Puccinia*, the leaf rust pathogen of barley involving five different stages of spores (aeciospores, urediniospores, teliospores, basidiospores and pycniospores) (Kolmer 2013).

As described above, rust diseases are known on barley for a long time. The incidence of leaf rust epidemics has increased in the past 15-20 years, especially in Europe (Clifford 1985). In Australia, there were minor reports of leaf rust epidemics prior to 1978 (Cotterill et al. 1992a), but epidemics during five years from 1978 to 1992 have been reported (Cotterill et al. 1992b). Melville et al. (1976) reported yield losses of 17-31% in the United Kingdom, in non-treated field trials opposed to those treated with fungicides. Two major epidemics were reported in North America, Mexico, and South America in 1989 and 1990 (Murray et al. 1998). Therefore, leaf rust resistance genes have become one of the main research objects, with great economic importance.

1.4 Resistance sources and mapping of leaf rust resistance genes in barley

Deployment of resistant cultivars is one of the most effective and economical means for controlling leaf rust (Burdon et al. 2014). Thus, research focusing on the identification and incorporation of new sources of resistance into breeding programs is of prime importance.

Generally, plants have two major types of defense mechanism against potential pathogens: Non-host resistance and host-specific resistance (Ávila Méndez and Romero 2017). Non-host resistance is known as a type of resistance which provides immunity against all isolates of a microorganism that is pathogenic to other plant species (Elmore et al. 2018). Near-nonhost resistance (Niks et al. 2011, Yeo et al. 2014) plays an important role in basic resistance breeding

of barley (Zellerhoff et al. 2010, Niks 2014, Yeo et al. 2014). In race-specific resistance, plants match single Mendelian resistance genes with the avirulence genes of different races of a pathogen. Rusts are very host specific, and therefore barley is not susceptible to most rust species infecting other cereals, and seems to be a near-nonhost to several non-adapted rust fungal species, such as *P. triticina* and *P. hordei-murini* (Jafary et al. 2008, Yeo et al. 2014).

Resistance is categorized in two types, i.e. qualitative (full, race-specific) and quantitative (partial) resistance (Elmore et al. 2018). Qualitative resistance is controlled by major resistance (*R*) genes that confer a hypersensitive reaction in response to races harboring the corresponding avirulence genes (Vale et al. 2001). This resistance has been detected against *P. hordei* in cultivated barley, landraces and exotic barley like *H. vulgare* ssp. *spontaneum* (Manisterski and Anikster 1994, Ivandic et al. 1998, Perovic et al. 2012). The second type of resistance is a quantitative resistance (partial resistance) which is in general controlled by several genes and is more robust than race-specific *R*-gene-based resistance (Corwin and Kliebenstein 2017). Partial resistance reduces the rate of disease (Vale et al. 2001). Therefore, partial resistance is presented by low infection effectiveness, long latent period, and low sporulation rate.

For decades, *R* genes against *P. hordei* were known as *Pa* genes after *Puccinia anomala*. In 1972, *Pa* genes were re-named to *Rph* genes (resistance against *P. hordei*), due to re-naming of the pathogen *P. anomala* to *P. hordei* (Moseman 1972, Ramage 1972). The *Rph* genes are numbered consecutively as *Rph1*, *Rph2*, etc. Currently, 26 *Rph* genes conferring qualitative resistance against *P. hordei* are known (Golegaonkar et al. 2009a, Hickey et al. 2011, König et al. 2012, Sandhu et al. 2012, Johnston et al. 2013, Sandhu et al. 2014, Yu et al. 2018). Among all known *Rph* genes, *Rph1* is the only *Rph* gene which has been isolated recently, using the newly developed cloning approach called Mutant Chromosome Sequencing (MutChromSeq) (Steuernagel et al. 2016) in combination with genetic mapping (Dracatos et al. 2018). *Rph* genes have been found to be active in barley seedlings and adult plants (Golegaonkar et al. 2009b, Golegaonkar et al. 2010). Different methods (e.g. trisomic, morphological or molecular analyses) have been used to localize *Rph* genes on all barley chromosomes (Chelkowski et al. 2003, Weerasena et al. 2004, Hickey et al. 2011, Sandhu et al. 2012).

Twenty-six different loci representing leaf rust resistance genes have been identified. *Rph1* was the first resistance gene identified in the cultivar *Oderbrucker* (Waterhouse 1948) and *Rph26* was most recently identified by Yu et al. (2018) in *H. bulbosum* introgression line 200A12. In 1971, Tuleen and McDaniel (1971) located *Rph1* (*Pa1*) on chromosome 2H using trisomic analysis. Borovkova et al. (1997) described the gene *RphQ*, which was mapped to the centromeric region of chromosome 5HS and was identified in the Australian barley line Q21861. *RphQ* is allelic or closely linked to *Rph2* (*Pa2*) detected in Peruvian (Borovkova et al. 1997). The *Rph3* (*Pa3*) gene in the cultivar Estate was located on the long arm of chromosome 1H (Jin et al. 1993). In 1969,

McDaniel and Hathcock (1969) mapped *Rph4* (*Pa4*) in the barley cultivar Gold to chromosome 1H. Previously, Tan (1978) reported that *Rph5* is located on chromosome 3H. Later, this gene was mapped to the short arm of chromosome 3H by Mammadov et al. (2003). Parlevliet (1976) reported that *Rph5* and *Rph7* are closely linked and located on chromosome 3HS and independent of *Rph2* located on chromosome 5HS. Tuleen and McDaniel (1971) mapped *Rph7* in Cebada Capa on chromosome3H and Tan (1978) confirmed this via trisomic analyses. Using molecular markers *Rph7* was also mapped on chromosome 3HS in Cebada Capa (Brunner et al. 2000, Graner et al. 2000).

Borovkova et al. (1998) reported that the resistance gene in barley cultivar Triumph is *Rph9* (previously designated as *Rph12*) and is located on the short arm of barley chromosome 5H. The research performed by Borovkova et al. (1998) concluded that the leaf rust resistance alleles *Rph9* of HOR259 and *Rph9* of Triumph are alleles of the same gene and not independent genes as previously reported by Jin et al. (1993). Feuerstein et al. (1990) mapped *Rph10* on the long arm of chromosome 3H, and *Rph11* on chromosome 6H. Ivandic et al. (1998) mapped *Rph16* to the short arm of chromosome 2H. Weerasena et al. (2004) illustrated that the *Rph15* locus is likely allelic to *Rph16,* due to the lack of segregation in F_2 progeny derived from the two resistance sources. Research performed by Jin et al. (1996) identified a resistance gene in barley accession PI584760 that is different from *Rph1* to *Rph13.* Thus, the gene was named *Rph14* and was mapped on chromosome 2HS by Golegaonkar et al. (2009). Origin and chromosomal location of all 26 *Rph* genes are presented in Table 1.

Table 1. Chromosomal location and origin of *Rph* genes.

Gene symbol	Chromosome localisation	Origin/Line	Reference
Rph 1	2H	Oderbrucker	(Tuleen and McDaniel 1971, Tan 1978)
Rph 2	5HS	Peruvian	(Borovkova et al. 1997, Franckowiak et al. 1997)
Rph 3	7HL	Estate	(Jin et al. 1993, Park 2003)
Rph 4	1HS	Gold	(McDaniel and Hathcock 1969)
Rph 5	3HS	Magnif 102	(Mammadov et al. 2003)
Rph 6	3HS	Bolivia	(Zhong et al. 2003)
Rph 7	3HS	Cebada Capa	(Brunner et al. 2000, Graner et al. 2000)
Rph 8	7HS	Egypt 4	(Borovkova et al. 1997)
Rph 9	5HS	Triumph	(Borovkova et al. 1998)
Rph 10	3HL	Clipper BC8	(Feuerstein et al. 1990)
Rph 11	6HS	Clipper BC67	(Feuerstein et al. 1990)
Rph 12	5HL	Trumpf	(Borovkova et al. 1998)
Rph 13	7H	PI531849	(Sun and Neate 2007)
Rph 14	2HS	PI584760	(Golegaonkar et al. 2009a)
Rph 15	2HS	Hordeum spontaneum	(Weerasena et al. 2004)
Rph 16	2HS	H. spontaneum/HSp.680	(Ivandic et al. 1998)
Rph 17	2HS	H. bulbosum/crosses between 81882 and 'Vada'	(Pickering et al. 1998)
Rph 18	2HL	cross between H. vulgare cv 'Emir' and a H. bulbosum HB2032	(Pickering et al. 2000)
Rph 19	7HL	Prior	(Park and Karakousis 2002)
Rph 20	5HS	Australian barley cultivar Flagship	(Hickey et al. 2011)
Rph 21	4H	Ricardo	(Sandhu et al. 2012)
Rph 22	2HL	H. bulbosum introgression line 182Q20	(Johnston et al. 2013)
Rph 23	7HS	Russian landrace LV-Taganrog	(Singh et al. 2015)
Rph 24	6H	H. vulgare/ND24260-1	(Ziems et al. 2017)
Rph 25	5HL	H. vulgare/Chinese barley variety 'Fong' Tien/Australian cultivar 'Baudin'	(Kavanagh et al. 2017)
Rph 26	1HL	H. bulbosum introgression line 200A12	(Yu et al. 2018)

Since novel sources of effective leaf rust resistance are rare in cultivated barley, there is a high demand for the identification and transfer of new effective resistances from wild barley (*Rph15* and *Rph16*). Resistance from wild barley are rather common in these species (Jin et al. 1995). In this regard, hypersensitive resistance controlled by single dominant genes, inherited in a simple Mendelian manner, were extensively used in breeding programs. They can be easily recognized and incorporated into adapted germplasm (Weibull et al. 2003). However, since newly occurring leaf rust races may overcome these genes in a short period of time, the deployment of race-specific *Rph* genes has not provided long-lasting robust resistance (Niks 1982, Steffenson et al. 1993). Breeding strategies aim at providing a more durable resistance in cultivars by pyramiding several

Rph genes, developing isogenic multi-lines, and combining effective *Rph* genes into lines having partial resistance (Jin et al. 1995, Dreiseitl and Steffenson 1999).

1.5 Histology of race-specific resistance

Biotrophic fungi (like rust pathogens) colonize the intercellular space of their host leaves and differentiate feeding structures, i.e. haustoria, to absorb nutrients and to suppress the host defense mechanisms without disrupting its plasma membrane (Tang et al. 2018). There are two main strategies that plants use to defend the attack and growth of biotrophic fungal pathogens, including penetration resistance and programmed cell death (PCD) resistance (Solomon Abera 2016). In general, phenolic compounds, lignin, reactive oxygen species, and proteins are thought to act as a physical barrier to halt penetration by the fungal penetration pegs and prevent the formation of haustoria (Underwood and Somerville 2008). Effective defense is largely due to programmed cell death (PCD) in the host, and to associated activation of defense responses including the activation of multiple signaling pathways and transcription of specific genes that limit pathogen proliferation and/or disease symptom expression (Ponce de León and Montesano 2013). Defense responses on the other hand, can regulate diverse chemical pathways like secondary metabolites production (Chowdhury et al. 2017). Two defense responses, which are commonly begin with gene-for-gene recognition of the pathogen are considered as hallmarks of gene-for-gene resistance (Knepper and Day 2010). One is a rapid production of reactive oxygen intermediates called the oxidative burst (Torres et al. 2006) and the other is a form of programmed cell death known as the hypersensitive response (HR) (Singh and Upadhyay 2013). For these two responses, which are mostly implicated in race-specific resistance, pathogens carry single dominant avirulence genes (*Avr*), the products of which are recognized by plants that carry corresponding resistance (*R*). Direct or indirect interaction of Avr and R proteins result in an incompatible interaction. In contrast, in the absence of the *Avr* gene in the pathogen and/or of the *R* gene in the host, the interaction is compatible (Solomon Abera 2016). So far many *R* and avirulence genes have been identified related to barley leaf rust (Kavanagh et al. 2017). The *R* genes comprise several major groups, of which the largest is the nucleotide binding site–leucine rich repeat (NBS-LRR) class (Elmore et al. 2011). The *Rph1* gene is a *R* gene against the barley leaf rust, carrying the NBS-LRR domain which has been isolated using the Mutant Chromosome Sequencing (MutChromSeq) (Dracatos et al. 2018). Thus, the interest in plant *R* genes is nurtured by the fact that such genes will play a crucial role during pathogenic development and defence mechanism.

1.6 The barley genome

Beside its agricultural relevance, barley is considered as an experimental model for other species of the *Triticeae* tribe, including wheat and rye (Hayes and Szűcs 2006, Schulte et al. 2009). In comparison to other crops, barley has several advantages. The spring form has a relatively short life cycle of around 15 weeks (Watson et al. 2018). Barley is an autogamous species with rarely occurring cross-pollination (Abdel-Ghani et al. 2004). The advantage of barley in genetic studies is due to its diploid nature, the low number of large chromosomes, self-pollination, easy hybridization, high degree of natural and easy inducible variation (Qi et al. 1996). The large genome size of approximately 5.3 Gbp is the only disadvantage of barley in genetic studies (Bennett and Smith 1976). The genome of barley is forty times larger than the Arabidopsis genome (125 Mb), nineteen times larger than the Brachypodium genome (272 Mb), thirteen times larger than the rice genome (389 Mb) and seven times larger than the sorghum genome (736 Mb) (Dolezel and Bartos 2005), but three times smaller than the wheat genome. The contiguous reference genome sequence of barley was recently published by Mascher et al. (2017) using hierarchical sequencing in combination with novel algorithm developed by company NRG (https://www.nrgene.com/). The number of high-confidence genes in barley has been reported to be 29,944 which were directly associated to pseudomolecules of specific chromosomes of the barley genome (Mascher et al. 2017).

1.7 Barley genomic resources

Nowadays, significant improvement in genomic resources for barley facilitate marker saturation as well as the procedure of chromosome walking (Perovic et al. 2018). During last decades, several genetic linkage/consensus maps have been published for barley. These resources contain different marker systems from hybridization-based restriction fragment length polymorphism (RFLP) (Graner et al. 1991, Kleinhofs et al. 1993) markers to PCR-based simple sequence repeat (SSR) markers (Ramsay et al. 2000, Varshney et al. 2007b), plus microarray-based single nucleotide polymorphism (SNP) markers (Close et al. 2009, Comadran et al. 2012, Bayer et al. 2017). In addition, numerous transcript-derived genetic maps were developed based on the improvement of barley genomics. For instance, Kota et al. (2003) developed 180 SNPs of which 72 could be mapped. Rostoks et al. (2005) developed and mapped 333 SNPs in barley. Later, 258 SNPs from 1,032 Expressed Sequence Tag (EST)-based markers were integrated to a barley consensus map by Stein et al. (2007). Two years later Close et al. (2009) reported that 3,072 EST-derived SNPs were placed on two Illumina Golden Gate assays (Illumina Inc., San Diego, USA) BOPA1 and BOPA2 (Barley Oligonucleotide Pooled Assay) which enabled the development of a consensus map with 2,943 integrated SNPs. This was the first Illumina array which has been

developed (Close et al. 2009, Munoz-Amatriain et al. 2011). Sequence contigs and high-density maps also were used to predict a virtual linear order of the barley genes. Mayer et al. (2011) developed a gene map of barley using the so-called genome zipper (GZ) approach via assembling 86% of the barley genes in a putative linear order. The second generation of the Illumina barley array platform, the 9K iSelect chip, was constructed on sequence polymorphisms in 10 different cultivated barley genotypes, containing 7,864 SNPs covering the former BOPA markers (2,832 SNPs) and 5,010 new SNPs (Comadran et al. 2012). Besides this, a number of 10 million SNPs derived from population sequencing (POPSEQ) were used to order and genetically anchor the barley physical map comprising more than 65,000 BAC clones (Ariyadasa et al. 2014). It enabled the improvement of genetically ordering the contigs from a whole-genome shotgun (WGS) assembly of the barley cultivar Morex. Silvar et al. (2015) evaluated the Genome Zipper and POPSEQ at seven loci with higher genetic resolution, which resulted in a higher accuracy in comparison to a newly developed consensus genetic map, respectively. The exome capture platform is another genomic resource, which is used in resequencing studies and gene isolation in barley (Mascher et al. 2013b, Mascher et al. 2014). Consensus maps as an additional genomic resource were constructed using 13 mapping populations and different Illumina platforms like the 9K Infinium iSelect and Illumina BeadXpress Arrays; suggesting an excellent opportunity to increase marker density for better comparison of genomic regions between QTL and association genetics studies (Silvar et al. 2015). More recently, the third Illumina barley array, the 50K Infinium array, with 44,040 SNPs markers is available (Bayer et al. 2017). In addition to the above mentioned resources, the barley reference genome sequence (Mascher et al. 2017) enables efficient marker development and the investigation of genetic diversity in barley and related species. In summary, a physical and genetic map of barley with whole genome sequence information (IBSC 2012, Mascher et al. 2017) combined with the above mentioned resources can efficiently be used for breeding purposes and facilitate an enhanced gene isolation in barley.

1.8 Novel sequencing technology

The advent of Sanger sequencing was revolutionary as it allowed genome sequencing for the first time (Sanger and Coulson 1975). A second revolution came when next-generation sequencing (NGS) technologies were developed driving down sequencing costs, increasing the sequence capacity and making the whole-genome sequencing and re-sequencing of crops feasible (Pettersson et al. 2009). Major advantages of NGS technologies are that they do not require bacterial cloning of DNA fragments and electrophoretic separation of sequencing products (Morey et al. 2013). However, NGS methods have several drawbacks; most notably their short read length. The development of NGS took place in late 20th and early 21st century. Next-generation

sequencing consist of different platforms including: Roche/454, Illumina/Solexa and Life/APG SOLiD (Hodzic et al. 2017). In 2007 genome analyser was released by Illumina, a company which introduced a sequencing-by-synthesis approach that is even today a staple of whole genome sequencing. It was capable to sequence 1Gbp in a single run. During the last five years, Illumina has developed Mi-Seq and Hi-Seq platforms. The Mi-Seq platform can sequence up to 15 Gbp, while Hi-Seq systems achieves up to 600 Gbp.

Shortly after the appearance of NGS, the third generation sequencing (TGS) technologies emerged (Hayden 2009). The most unique features of TGS are single-molecule sequencing (SMS) and sequencing in real time (Schadt et al. 2010). The first true TGS technology was released in 2011 by Pacific Biosciences (PacBio) and is termed 'single-molecule real-time' (SMRT) sequencing. The average length reads in SMRT is 10-15 Kb up to maximum read length of >80 Kb (Sakai et al. 2015). More recently in 2014, Oxford Nanopore Technologies (ONT) introduced nanopore sequencing with variable in read length (read lengths are limited only by the molecule lengths in the sample) (Jain et al. 2015). In addition to the absence of PCR amplification and the real-time sequencing process, an important feature of SMRT and nanopore sequencing is the production of long reads. In 2014 as an alternative to the methods developed by PacBio and ONT, Illumina introduced a library preparation kit for 'synthetic long reads' (SLRs). One year later 10X Genomics introduced a micro fluidics variant of SLR with much higher partitioning capacity. In Illumina SLR, DNA is sheared into ~10-kb fragments, while the 10X Genomics system uses natural fragments of arbitrary size up to ~100 kb (Van Dijk et al. 2018).

When it comes to application of above mentioned technologies to plant species, the broadest and most prominent is whole genome sequencing (WGS) aiming to the full sequence of plant genomes (Hodzic et al. 2017). New sequencing technology have also been widely used in Complexity Reduction of Polymorphic Sequences (CRoPS), Restriction site Associated DNA (RAD) (Baird et al. 2008), Multiplex Shotgun Genotyping (MSG) (Andolfatto et al. 2011), Sequence Based Polymorphic marker technology (SBP) (Sahu et al. 2012), Genotyping by Sequencing (GBS) (Elshire et al. 2011) and gene isolation. RAD-seq and GBS have already been proven to be effective for next generation plant breeding (Yang et al. 2012, Glaubitz et al. 2014). At the same time above mentioned sequencing techniques have been used in the generation of several thousands of markers of which SNPs are the most abundant in genomes (Ganal et al. 2019). The NGS technologies are also being applied for targeted re-sequencing to identify domestication related genes by comparing the genome of crop species and their wild relatives (Henry 2012). More recently, the combination of mutational genetics and NGS allows rapid targeting and isolation of all type of genes. For instance, resistance gene enrichment sequencing (RenSeq) (Andolfo et al. 2014), and three newly developed methods including Mutant Chromosome Sequencing (MutChromSeq) (Steuernagel et al. 2017a), association genetics with *R* gene enrichment

sequencing (AgRenSeq) (Arora et al. 2019) and Mutant resistance gene enrichment sequencing (MutRenSeq) (Steuernagel et al. 2017b). Similarly, RNA-Seq analyses is also a cost-efficient approach to identify a mutated gene in a group of allelic mutants (Hansson et al. 2018). In another method so-called bulked segregant RNA-Seq (BSR-Seq) analyses, samples from mutant and non-mutant plants of a segregating F_2 population are combined into two separate pools and subjected to RNA-Seq (Liu et al. 2012).

1.9 Genetic maps and molecular markers

Genetic markers are important tools in the field of plant breeding. DNA markers have promoted genetics, genomics and breeding in a wide range of plant species, including barley, through their use in the construction of linkage maps, which are a useful tool for marker-assisted selection (MAS), association genetics analysis and QTL analysis (Nadeem et al. 2018). Genetic markers are commonly grouped into two categories, I: classical markers and II: DNA/molecular markers. Morphological, cytological and biochemical markers are types of classical markers. An Example for biochemical markers is the hordein composition which is an effective marker of traits which are localized on the short arm of the barley chromosome 1H like *M1* loci for resistance to powdery mildew (*Erysiphe graminis* f.sp. *hordei*) (Perovic et al. 2009). Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSRs), Single-Nucleotide Polymorphism (SNP), Expressed Sequence Tag (EST) and Diversity Arrays Technology (DArT) markers are the most common DNA markers (Jiang 2013). Markers positioned close to the gene of interest on the same chromosome are known as linked markers and are useful for quantitative trait locus (QTL) detection, marker-assisted selection, and marker-assisted cloning (Mohan et al. 1997, Morton 2005).

Different molecular marker types emerged in the late 1980s–late 1990s and were used to develop genetic maps (Shin et al. 1990, Graner et al. 1991, Heun et al. 1991, Kleinhofs et al. 1993, Ramsay et al. 2000). Based on these studies, up to 568 SSR-based markers were distributed on the seven barley chromosomes (Ramsay et al. 2000). A decade later, the number of identified markers had increased and several high density maps based on 1000–3500 markers were published (Rostoks et al. 2005, Wenzl et al. 2006, Hearnden et al. 2007, Marcel et al. 2007, Stein et al. 2007, Varshney et al. 2007a, Potokina et al. 2008, Sato et al. 2009, Szűcs et al. 2009). High throughput genotyping in barley was first introduced in 2006 with the development of two Illumina GoldenGate assays (Fan et al. 2003) that featured 1,572 SNP markers each. In 2009, Close et al. (2009) explored more than half a million of EST sequences available at the public dbEST database, which had been obtained from several barley cultivars (Close et al. 2009). Complemented with sequenced PCR amplicons derived from genomic sequences, approximately 22,000 SNPs were identified, of which

3072 were selected for the production of the Illumina GoldenGate oligonucleotide pool assays named BOPA1 and BOPA2. An improved genetic map was developed with the same SNP platform on 10 mapping populations and 2994 SNP loci were mapped to 1163 unique positions with a total length of 1137 cM (Munoz-Amatriain et al. 2011). The Illumina 9K iSelect array included 7,864 SNPs discovery in Illumina RNAseq data from 10 UK elite cultivars (Comadran et al. 2012). More recently, the Illumina 50K iSelect chip and population sequencing (POPSEQ), resulted in identification and mapping of number 44,040, and over 11 million SNPs respectively (Mascher et al. 2013a, Bayer et al. 2017).

1.10 Gene identification and positional cloning in barley

In general, to study the gene functions two basic approaches can be conducted i.e. forward genetics and reverse genetics. Forward genetics refers to a process where studies are initiated to determine the genetic underpinnings of observable phenotypic variation (Hricová et al. 2010). In reverse genetics, a specific gene or gene product is disrupted or modified and then the phenotype is scored (Zakhrabekovaa et al. 2013). This approach aims to test the impact of a gene by direct modification of the gene, resulting in a change or complete loss of function. In barley, this method attains importance for validating gene functions to confirm the identified candidate genes.

In barley, map-based cloning became a standard forward genetics approach for the isolation of disease resistance genes (Stein and Graner 2005). The procedure of map-based cloning starts with the construction of a segregating mapping population, derived from a cross between two genotypes with contrasting phenotypes. The segregation of the phenotype indicates if the trait is inherited in a monogenic or polygenic manner, i.e. if it is controlled by one or multiple genes. The information from the analysis of a segregating population with genome wide markers allows the identification of the linkage group carrying the gene of interest resulting, in the identification of suitable flanking markers for high-resolution mapping. In a next step, further markers are needed to saturate the target interval. The purpose is to discover markers in close linkage to the gene of interest.

A large number of meiotic events is necessary for a sufficient genetic resolution to identify recombination events in close vicinity to the target gene (IBSC 2012). Although high resolution mapping provides deep information for the target locus, the uneven distribution of crossovers along chromosomes (IBSC 2012) and the large variation in the genetic/physical ratio across the genome (Kunzel et al. 2000) often hampers high-resolution genetic dissection. In barley, peri-centromeric regions (pCENR) comprise at least 48% of the physical genome but harbour only 14–22% of the total barley gene content (Mascher et al. 2017). The other extreme are hotspots of high recombination rates in telomeric regions (Bhakta et al. 2015). In case of the *Ryd3*, which is located

in a centromeric region, the physical/genetic ratio has been estimated at 14–60 Mb/cM, while the genome-wide average is 4.4 Mb/cM (Lüpken et al. 2014). At the *rym4*/*rym5* locus, the ratio of physical to genetic distances was in the range between 0.8 and 2.3 Mb/cM, although the gene has been mapped in the telomeric region of chromosome 3H (Stein et al. 2005). Significant improvement in genomic resources together with sequencing technologies for barley facilitates mapping-by-sequencing as well as marker saturation (Mascher et al. 2014). Today, mapped genes can be assigned precisely to the barley reference genome, giving the information about putative candidate genes (Yang et al. 2014).

1.11 Physical mapping

Physical maps are pivotal for map-based cloning of genes in large, repetitive genomes to overcome the complexities. Since genetic map distance are not directly related to physical distances, physical mapping is required to define the locations of markers in order to get information on distances in base pairs. Wild diploid wheat, *Aegilops tauschii* the ancestral donor species of the D genome of *Triticeae aestivum* was the first *Triticeae* species subjected to whole genome physical map construction (Stein 2009). In recent years, lots of efforts were undertaken for whole genome sequencing of important cereals such as barley (Mayer et al. 2012, Mascher et al. 2017). However, due to the large size of the barley genome (5.3 Gb) and its high content of repetitive DNA (80%), this is not an easy task. In 2012, the International Barley Genome Sequencing Consortium (IBGSC) set out an international project to construct a high-quality physical map of the barley cultivar Morex using high-information-content fingerprinting and contig assembly of 571,000 bacterial artificial chromosome (BAC) clones from six independent BAC libraries (IBSC 2012). With their work on the barley genome, the IBSC succeeded in producing the first high-quality reference genome for barley (IBSC 2012). In this project, 5.1 Gb, i.e. more than 95% of the barley genome was represented in the physical map. Out of these, roughly 1.9 Gb (48% of the genetically anchored physical map) was assigned to centromeric regions. 15,719 “high-confidence” and 53,220 “low-confidence” genes which could be directly associated with the genetically anchored physical map were reported (IBSC 2012). In 2017, Mascher et al. (2017) released the reference barley genome sequence of seven barley chromosomes. This information provides an indispensable reference for genetic research and breeding. Access to a whole genome physical map allows efficient isolation of genes that underpin important traits like disease resistance genes.

1.12 Objectives

This PhD thesis is based on the work of König et al. (2012), who mapped the major resistance gene *Rph*$_{MBR1012}$ in the Yugoslavian barley landrace MBR1012. Using a population comprising 91 doubled haploid (DH) lines (Scarlett × MBR1012), the gene was mapped in the telomeric region of the short arm of chromosome 1H between the closest linked markers GBMS187, GBS546 distal (0.8 cM) and GBS21 proximal (6.0 cM).

Based on this information, the main objectives of the present PhD thesis are to (i) develop a high-resolution mapping population for the *Rph*$_{MBR1012}$ resistance gene, (ii) saturate the locus using all available state-of-the-art genomic resources i.e. GBS, the 50K Infinium chip and the barley reference genome, (iii) anchor the genetic map to the barley reference sequence, (iv) characterize the putative candidate rust resistance genes by allele specific re-sequencing, (v) test the developed markers for their diagnostic value, and (vi) get histopathological information on race-specific resistance encoded by *Rph*$_{MBR1012}$.

2 Material and methods

2.1 Plant material and construction of high resolution mapping population

A high resolution mapping population was constructed comprising 4775 F_2 plants originating from crosses between five DH lines, namely, the resistant DH-lines DH3/6 and DH3/127 and the susceptible DHs DH3/9, DH3/62 and DH3/74, which were derived from the original cross between the parental line MBR1012 (resistant) and Scarlett (susceptible). Based on these 5 DH lines, 4 crosses were conducted i.e. DH3/74 (S) × DH3/6 (R), DH3/74 (S) × DH3/127 (R), DH3/6 (R) × DH3/9 (S), DH3/62 (S) × DH3/127 (R) (Table 9). In order to identify recombinants, F_2 plants were analysed using the co-dominant flanking SSR markers, QBS94 (distal) and QBS113 (proximal) according to (Perovic et al. 2013). From the F_2 population, non-recombinant plants were discarded and heterozygous recombinant plants were used to identify homozygous recombinant inbreed lines (RILs) (Figure 5). For F_2 plants being heterozygous recombinant in target interval, twelve seeds were sown in 96 Quick pot plates. Genomic DNA of 10 days old plantlets was extracted for F_2 and F_3 analyses according to Dorokhov and Klocke (1997). Next, plants were transferred to vernalization (4°C) for 4-6 weeks and then to 15 °C for acclimatization for one week. The selected homozygous recombinant plants were transferred into individual pots (14 cm diameter) and grown under greenhouse conditions with a temperature cycle of 20°C/18°C (day/night) and a photoperiod of 16h light/8h dark and 50% humidity. These plants were selfed and single seed descendant F_4-plants underwent phenotyping for disease resistance. By this approach, a high resolution mapping population of 537 was constructed and used for marker saturation and resistance testing.

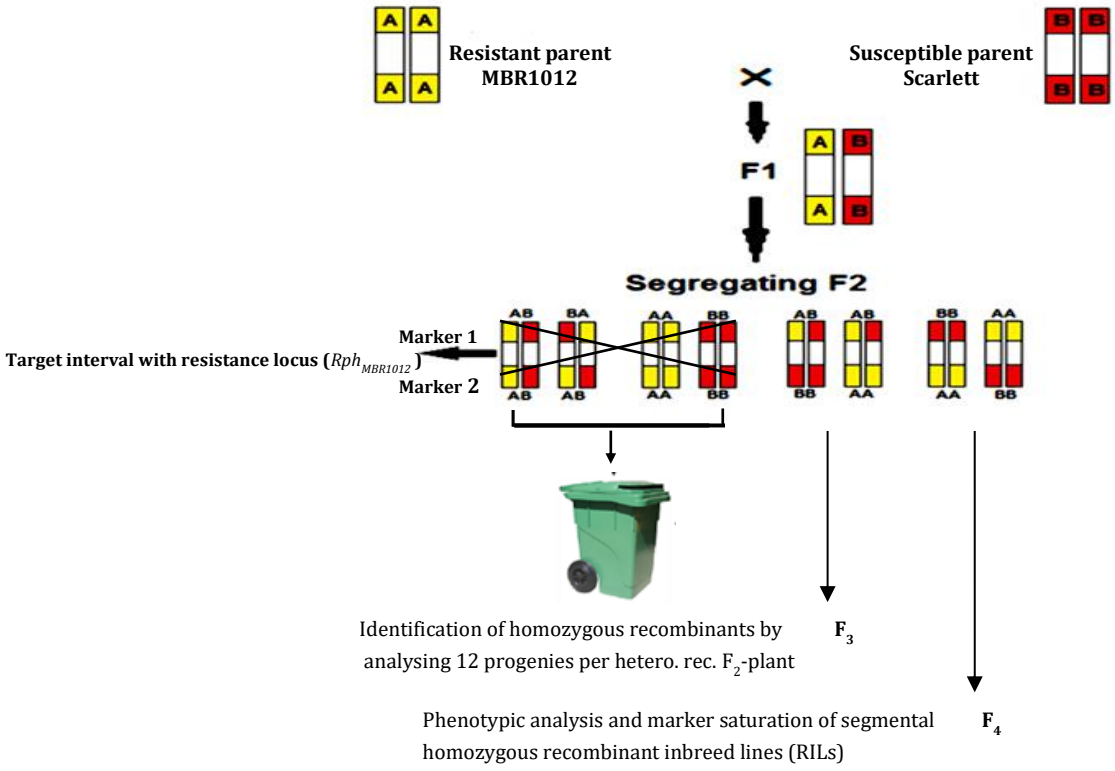

Figure 5. Schematic overview of population development for mapping of the *Rph*$_{MBR1012}$ locus.

2.2 Disease assessment

2.2.1 Propagation of urediniospores of *Puccinia hordei*

The leaf rust isolate "I-80" was propagated by artificial inoculation of seedlings at the two-leaf stage of *Hordeum vulgare* cultivar "Grossklappige", which showed the highest susceptibility to the most virulent isolates of *Puccinia hordei*. Seedlings were inoculated with a mixture of fresh spores and white clay (Laborchemie Apolda, Germany), (1:3). Inoculated plants were covered with plastic for 24 h to create a moist environment favorable for the infection and grown at 18°C. After 15 days, rust urediniospores were harvested and used to inoculate RILs.

2.2.2 Macroscopic Screening

Phenotypic tests were conducted under controlled greenhouse condition. Resistance tests were carried out by inoculation of whole leaves of segmental homozygous F_4 RILs along with the two *H. vulgare* parental lines, i.e. MBR1012 (resistant), Scarlett (susceptible) and susceptible (DH3/62) and resistant (DH3/127) DH-lines as well as the cv. Grossklappige as a control. For

each RIL three seedlings were tested, preferentially in two replicates, depending on the availability of sufficient seeds. Plants were sown in 96 Quick pot trays and were grown with a temperature cycle of 20°C/18°C (day/night), 50% relative humidity and a photoperiod of 16 h (360 µM $m^{-2}s^{-1}$ photosynthetic photon flux density). 10 days old plantlets were sprayed with Tween20 in order to increase the efficiency of inoculation on the leaf surface. Ten milligrams of fresh spores per 100 plants were mixed with white clay (Laborchemie Apolda, Germany), (1:3) and were scattered to the plants using a special pump according to (Ivandic et al. 1998). Next, the inoculated plants were placed in a growth chamber (18°C) and covered with plastic for 24 h providing a moist environment for successful infection. Infection types (IT) were generally assessed 10 and 13 days post inoculation (dpi). Infection types (IT) were generally evaluated according to Levine and Cherewick (1952). ITs, "0" (no visible uredinia), "0c", "0n" (hypersensitive reactions with necrotic/chlorotic 'flecks'), "1" (a very small uredinia in clearly developed necrotic areas), "2-" (small and little sporulated pustules surrounded by necrotic/chlorotic areas), or "0-2-" (a flecks range of 0 to 2-) were considered as resistant, whereas those displaying ITs, "2+" (moderate to quite large uredinia with or without chlorosis), and "3" (well sporulated pustules without chlorosis) were considered a susceptible. Segregation of resistant and susceptible plants was analysed using the Chi-square (X^2) tests for goodness-of-fit to the expected Mendelian segregation ratios (Griffiths et al. 2000).

$$X^2 = \sum \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}}$$

2.2.3 Microscopic investigation of fungal development

To evaluate fungal pre- and post-invasive development, three different staining methods including 3,3'-diaminobenzidine (DAB) (Daudi and O'Brien 2012), Calcofluor White M2R (Rohringer et al. 1977), and Propidium iodide (Jones et al. 2016) were applied simultaneously. All chemical substances were provided by Sigma-Aldrich, Munich, Germany.

For 3,3'-diaminobenzidine (DAB) and Calcofluor White M2R staining, 1.5 cm of flag leaves were cut at five time points; 24, 48 and 72 hpi (hours post inoculation) as well as 7 and 8 dpi (days post inoculation). Samples were placed in reaction tubes (2 ml) and were incubated in 1.5 ml of the DAB solution (1 mg ml^{-1} aqueous DAB, pH 3.8 containing 0.05% (v/v) Tween20) in the dark at room temperature overnight. Keeping the samples overnight in DAB solution caused the solution to equally disperse within the entire leaf. Fungal cell walls were stained using Calcofluor White M2R (Rohringer et al. 1977). After the DAB stain, leaves were washed twice with deionized water

and transferred to reaction tubes (2 ml volume) which contained 1.5 ml of a lactophenol/ ethanol (1:2 v/v). The lactophenol/ethanol solution was prepared by mixing 100 g phenol in a solution of 50 ml lactic acid, 100 ml glycerol, 50 ml deionized water and 480 ml of ethanol. After an incubation time of 2 h, samples were boiled for 10 min at 110°C. 1.5 ml of a solution of ethanol and water (33.3% ethanol, 66.6% deionized water (v/v)) was then added and agitated at 400 g for 15 min at room temperature. The ethanol/H_2O solution was discarded and 1.5 ml of 0.05 M sodium hydroxide was added. Samples were shaken for 15 min, washed with distilled water (400 g; 15 min; RT) and placed into 0.1 M Tris-HCl and incubated at RT for 1-2 h. Finally, 0.2% (w/v) Calcofluor white M2R solution was added and discarded after 5 min of incubation. Leaves were washed 4 times with sterile water, each for 10 min. Stained samples were mounted on microscopy slides and embedded in a glycerol/water solution (1:1 v/v).

In an independent experiment, 1.5 ml of propidium iodide solution (10 µg/ml) was incubated on 7 dpi cut leaf samples overnight at RT and mounted as above.

Chitin fluorescence was visualized using an Axioskop 50 fluorescence microscope and analysed with an Axiocam MRc and the software package Axiovision 4 (Carl Zeiss AG, Jena, Germany). Calcofluor white M2R, samples were analysed using the filter set 02 (excitation filter G 365, beam splitter FT 395, and barrier filter LP 420), autofluorescence within plant tissue was recorded using the filter set 05 (excitation filter BP 400-440, beam splitter FT 460, barrier filter LP 470).

2.3 DNA extraction

In order to select the homozygous recombinant plants, leaf samples of 10 days old $F_2/_3$ plantlets were cut for DNA extraction. Genomic DNA was extracted via quick and dirty method developed by Dorokhov and Klocke (1997) using 96 well plates. All extracted DNA samples were tested with two flanking markers BOPA1_8670/QBS94 and BOPA1_7372/QBS113. In a second step, after selecting the homozygous recombinant plants, genomic DNA from 5-weeks-old F_4 plants (RILs) was extracted using a Cetyltrimethyl Ammonium Bromide (CTAB)-based DNA isolation approach (Stein et al. 2001) and was dissolved in 80 µl of 1 x TE buffer (1 M Tris-HCl, and 0.1 M EDTA), (pH 7.5-8.0). The quantity and quality of the obtained DNA was determined by using a NanoDrop Spectrophotometer (PEQLab, Erlangen, Germany) following manufacturer's instructions. Concentration was measured based on absorbance at 260 nm, and purity was checked by the ratio of absorbance at 260 and 280 nm. The quality of the extracted genomic DNA also was checked by electrophoresis on 1% TAE agarose gels. The DNA was stored at -20°C for long term storage, after taking an aliquot and adjusting to (20 ng/µl) for use in PCR applications, as well as for genotyping on the 50K illumina array and GBS.

2.4 Polymerase chain reaction (PCR)

Standard PCR amplification was performed in a reaction volume of 10 µl, containing 20 ng/µl template-DNA, 0.25 µl forward (1 pmol/ µl) and reverse (10 pmol/ µl) primers, 0.08 units of FIREPol®DNA polymerase (5U/µl), (Solis BioDyne, Tartu, Estonia), 1 µl of 10X PCR buffer BD (Solis BioDyne), 1 µl of 25 mM $MgCl_2$ and 0.2 µl of 10 mM dNTPs (Fermentas, Schwerte, Germany). M13 tailed forward primers were used, so that 0.1 µl of M13 primer (10.0 pmol/ µl) (5'-CACGACGTTGTAAAACGAC-3') labelled with 5' fluorescent dye was added to the reaction mix. Details of the PCR reaction mix has been presented in appendixes 1 and 2. A touch-down PCR program was used with a GeneAmp 9700 thermal cycler (Applied Biosystems, Darmstadt, Germany): first denaturation at 94°C for 5 min followed by 12 cycles at 94°C for 30 s, annealing at 62°C to 56°C (-0.5°C/cycle) for 30 s, extension 30 s at 72°C, and then proceeded with 35 cycles 94°C for 30 s, 56°C for 30 s, 72°C 30 s, and followed by a final extension at 72°C for 10 min. Regarding to different primer sets and different experiments, the respective reaction volume and PCR cycling condition were changed with minor modifications. The PCR products were separated by 1.5 % agarose gel electrophoresis. Detailed information of the optimized PCR program corresponding to each marker used in this study is given in appendixes 3 and 4.

2.5 Agarose gel electrophoresis analysis

The PCR-amplicons were resolved by gel-electrophoresis. 1.5% (w/v) agarose (UltraPure™ Agarose, Invitrogen GmbH, Darmstadt, Germany) TBE gels (89 mM Tris-borate, pH 8.3; 2 mM Na2EDTA) (Sambrook et al. 1989). Two µl of ethidium bromide (0.5 µg/ml) were added to the gel before polymerizing. The Puc19 and 50bp DNA ladders (Fermentas GmbH, St. Leon-Rot, Germany) served as size standards. The gel was run in an electrophoresis chamber (Bio-Rad Laboratories GmbH, Munich, Germany) in 1X TBE buffer (Appendix 5) and were separated in an electric field of 95 V for 2 h to 3 h according to their fragment size. DNA was visualized under UV light and images were captured by an INTAS gel documentation system (Intas Science Imaging Instruments, Göttingen, Germany) together with the imaging software Quantity One (Bio-Rad, Munich).

2.6 Marker saturation

2.6.1 Markers derived from the genome zipper (GZ) and the 9K iSelect Illumina array

For marker saturation, initially two marker resources namely the genome zipper (GZ) (Mayer et al. 2011) and the 9K Infinium iSelect high density custom genotyping array (Comadran et al. 2012) were simultaneously implemented for random saturation of the large interval of about 8 cM (Perovic et al., personal communication). In total, six SSRs based markers from the pyrosequencing assay, three dominant present/absent markers, four size polymorphism markers (insertion/deletion polymorphisms (InDels) and 24 Cleaved Amplified Polymorphic Sequences (CAPS) markers were used for first marker saturation and were mapped on the 537 RILs (Tables 2 and 3). Size polymorphisms markers and SSRs were amplified in a total volume of 10 µl, according to (Perovic et al. 2013) and detected either as direct polymorphism on 1.5% agarose gel or by using ABI capillary system (Applied Biosystems 3130 Genetic Analysers). For ABI analysis, 0.1 µl of M13 primer (10.0 pmol/ µl) (5'-CACGACGTTGTAAAACGAC-3') labelled with fluorescent dye was added to the reaction mix. 1 µl of diluted PCR product was added to 14 µl of HiDi-Rox mastermix (1.4 ml Hidi and 6 µl Rox) in a total volume of 15 µl. Results were analysed by the software package GeneMapper v4.0 (Applied Biosystems, Darmstadt, Germany). In both methods of detection, all genotypes were compared to the parents MBR1012 and Scarlett.

Table 2. Primer pairs designed based on sequence information from the genome zipper (GZ) and 9K iSelect chip.

Marker/primer	Forward sequence	Reverse sequence
QBS94_BOPA1_8670_388_s22_as141	ATATACACATTTCCAGCAGCGA	AACCTTTTTGGTACAGCCTAGC
GBMS187	CGATGTTTATGATGGGAGGG	TTGTCTCCTCCCGTCAGC
GBR534	GACAAGGAGTTCAAGCTGCC	TGGTGCAATCCCATAACAGA
GBS564	GAGCAGTTGCCATGTGTTGC	GGCATGATGTCGAATAACGG
QBS2	AGCTGAATCCAACCCAACAC	AGTCGCAGAGCCACAAGTTC
QBS78_contig50849_s216_as1256	CTCAAGGACAGTGCGAATGA	TGTACACGTGACGGAGGAAG
QBS98_GZ29_M_contig_161159_s1073_as1968	CTCCTAACCGTAGCCAAGCC	TCCCCAACTCCCAAACACAC
QBS96_BOPA7174-365_contig127650_s31_as336	ACTTGATCGGCTCTTCCATC	CGCTTAGTCGCATTTCTGGT
QBS71_contig1031142_s338_as1388	CATCTCCCTCTTCTTGGTGG	TGGCATTGGTGGGTAATTTT
QBS99_GZ29_M_contig_161159_s90_as1968	AGCTACCTGCTAGGTTCCCT	TCCCCAACTCCCAAACACAC
QBS106_GZ59_M_contig_45711_s3862_as4545	GAGCTTTAGTTGGTGCAGCG	ATGGGGCTCCATTAAGTAGCTG
QBS110_GZ66_B_contig370643_s7768_as8867	GCATGGTCATATGCTTCTGGA	GGCTGCCTTTTTCTTGTTGAGA
QBS113_BOPA1_7372_139	CATTGATTGACACCACCAGC	TCATCCACTTCACACCTCCA

Table 3. CAPS markers designed based on sequence information of 9K iSelect markers and genome zipper (GZ).

CAPS marker/primer	Forward sequence	Revers sequence
QBS72_contig_1008249_s315_as1362	ATAGGATCGTTTCGGCTCCT	CATGGGCCAAGTGTATCAGA
QBS73_contig_1008249_s133_as1186	TGCAGCAACAAAAAGGAGAA	CGGATCGACAAGGATAAGGA
QBS74_contig_2160218_s20_as250	AGGTCAAGGAAAACAATCGC	CCGAAGAAGAAGGTGGTCAT
GBS626	CCAACTCTGGTGTCAATGGCT	GCAGTTGCCATGTGTTGCAG
GBS546	AACTCTCGTGTCAATGCCGA	CAACGTATTGCAAGGTCGCA
QBS75_contig_1019464_s215_as1264	TATGGCTGGAAAAGGTCTCG	CACATGCCTCGATCTCTCCT
QBS104_BOPA2_ctig_54745_s80_as349	CGATTCCTATGCCTGCAGAT	CTTGTATGCCGCATCCTTCT
QBS76_contig53937_s351_as1405	TGCAGGGAAGTAACATGCAG	AATCTTGCCCCTCGTTCTTT
QBS77_contig145384_s22_as1066	TGTAGCTGAAAACCTGGGCT	CGGATCAACGCATGTTATTG
QBS97_contig_1008249_s6_as1304	AGGGAGACGTTGATCACTGC	CGTCAGATTCGGGTTGAATC
QBS100_GZ54_M_contig_2547982_s282_as487	ACCGTGCCTCTCACAAACAA	TTCGTGCGCCGTTATTTGAC
QBS70_contig_64079_s49_as297	GAAATGCGGCCTTATGTTGT	ATAGAGGACACGCCGTCAAG
QBS95_30969_ctig_121098_s22_as316	TGCTCTCGTCATGGAGAAGA	CAATCATCGGGACTCAATCC
QBS101_contig_1019464_s115_as1400	CGGCCTGGAAGAACATTTAA	ACGCTTTCTTGGCACCTAAA
QBS102_contig_1019464_s164_as1479	TCTCCCATGCCAGCTAATTT	GGGATCCAAAGATTTCCCAT
QBS103_contig_1019464_s17_as1310	AAGAAGAAGAAGGGCAAGCC	GGCTTGCCCTTCTTCTTCTT
QBS105_3101_111_s6_as135	AGGGGATGGTTTGCCTTGTA	TGAGTCACTGGCTGCCGACAC
QBS79_contig121251_s111_as1150	CACTGGCTCAAGCTGCTACA	ACCTTCTCCCACACCTCCTT
QBS107_GZ59_M_contig_s45711_s976_as1572	ATGCTTCTGGCCTCCTCTTG	GTACCCGTCACAGTTGGAGC
QBS108_149683_ctig_224474_s8_as303	ATGGGTAGTCGGTGTAGCCA	TATCCACATGATCGAACCGA
QBS109_232577_ctig_1007221_s20_as318	TGACTCACAGATTGGCTTGC	ATATCACTCCTCGCTTTGCC
QBS80_contig247169_s465_as1513	ATGGCGTAGGCTGTTGAAAG	ATTGCAGATGCAGACTCCCT
QBS111_GZ67_BO_contig_66602_s8296_as_9813	AGGTACATCACTCCTCTCCCT	GGTGGCCACTGTGCTTTATCT
QBS112_GZ67_BO_contig_66602_s8931_as_s9813	CTGCCTCACCTTCTCTTTCTCT	GGTGGCCACTGTGCTTTATCT

2.6.1.1 Primer design

The primers used for marker development and saturation were designed using the online software Primer3 v. 0.4.0 (http://bioinfo.ut.ee/primer3-0.4.0/) (Koressaar and Remm 2007, Untergasser et al. 2012). Default parameters were used with the following modifications: length 20-22 bp, melting temperature 58-62°C and GC content was set within the range of 50%-60% and the product size to a range varied according to SNP position and detection method between 100 and 800bp.

2.6.1.2 Cleaved Amplified Polymorphic Sequences (CAPS) marker analysis

In order to convert the putative SNPs between parental lines into CAPS markers, sequences of around 20 bp length, containing a SNP, were used to find restriction sites and select appropriate restriction endonucleases (common enzyme, low price, high specificity under uniform standard incubation conditions), with the online tool NEBcutter v2.0 (http://tools.neb.com/NEBcutter2/) (Vincze et al. 2003). Digestion was performed in a 20 µl reaction volume containing 8 to10 µl of PCR product, 2 µl of the corresponding 10X buffer (New England Biolabs, Hitchin, UK), 0.1 µl of the appropriate enzyme (10 u/µl), (for enzymes with the concentration 5 U/µl 0.2 µl were used) and adjusted to final volume by adding 7.9 µl of ddH_2O (Carl Roth, Karlsruhe, Germany). The reaction mix was incubated for 2-3 hours in a water bath with a constant temperature according to the enzyme used in order to allow the digestion of allele specific PCR products according to the manufacturer's instructions (New England Biolabs and Fermentas) (see appendixes 6 and 7 for digestion protocol and condition). Two microliters of the digested PCR product mixed with 8 µl of 6X loading dye was loaded on a 1.5% agarose gel containing 0.5 µg ml^{-1} ethidium bromide. Electrophoretic separation of fragments was conducted in 1X TBE-buffer at 95 V for 2 h – 3 h. For CAPS GBS626, GBS546, QBS70, QBS74, QBS95, QBS100, QBS104, QBS105, QBS108 andQBS109 the m13 tail was used and digested fragments were separated using a capillary electrophoresis ABI PRISM® 3100 genetic analyzer (Applied Biosystems) according to Perovic et al. (2013).

2.6.2 Marker development from 50K and genotyping-by-sequencing (GBS)

2.6.2.1 50K iSelect genotyping

To saturate the target locus with additional molecular markers, the Illumina 50K array and Genotyping by Sequencing (GBS) was employed. In order to select putative markers from the 50K chip, the genomic DNA of three resistant lines (MBR1012, DH3/127, RILs: 11/1_107_06) and three susceptible lines (Scarlett, DH3/62, RILs: 9/4_192_12) were sent for genotyping to Trait Genetics, Gatersleben. Among all identified polymorphic SNPs between resistant and susceptible lines (highlighted with the red color in table 4), 19 selected ones located in the target interval were converted into Kompetitive Allele Specific PCR (KASP) assays (http://www.lgcgroup.com/), (Table 4).

Table 4. 50K Molecular markers located on chromosome 1H. (Red markers are located in the target interval and were used for maker saturation).

Marker	DH3/127_R	MBR1012_R	RIL-107-06_R	DH3/62_S	Scarlett_S	RIL192_12_S	Morex	chr	start	End
JHI-Hv50K-2016-97	C	C	C	A	A	A	A	chr1H	51263	51383
BOPA2_12_10420	A	A	A	C	C	C	C	chr1H	71604	71484
JHI-Hv50K-2016-127	C	C	C	T	T	T	T	chr1H	71999	72119
SCRI_RS_204276	T	T	T	C	C	C	C	chr1H	248471	248351
JHI-Hv50K-2016-137	A	A	A	T	T	T	T	chr1H	248672	248792
JHI-Hv50K-2016-141	G	G	G	A	A	A	A	chr1H	249631	249751
JHI-Hv50K-2016-157	A	A	A	G	G	G	G	chr1H	254019	254139
JHI-Hv50K-2016-170	C	C	C	G	G	G	G	chr1H	261909	262027
JHI-Hv50K-2016-211	C	C	C	T	T	T	T	chr1H	269782	269897
BOPA2_12_30653	G	G	G	A	A	A	A	chr1H	272757	272637
JHI-Hv50K-2016-237	C	C	C	A	A	A	A	chr1H	272930	273050
JHI-Hv50K-2016-256	C	C	C	A	A	A	A	chr1H	274127	274247
JHI-Hv50K-2016-270	T	T	T	G	G	G	T	chr1H	277172	277290
JHI-Hv50K-2016-392	C	C	C	T	T	T	T	chr1H	474901	475019
BOPA1_7174-365	C	C	C	G	G	G	C	chr1H	479052	479292
JHI-Hv50K-2016-468	G	G	G	A	A	A	G	chr1H	479634	479754
JHI-Hv50K-2016-745	A	A	A	G	G	G	A	chr1H	945971	946091
JHI-Hv50K-2016-847	A	A	A	G	G	G	G	chr1H	971563	971683
JHI-Hv50K-2016-877	T	T	T	C	C	C	C	chr1H	973709	973829
BOPA2_12_30969	G	G	G	A	A	A	G	chr1H	977855	977975
JHI-Hv50K-2016-912	C	C	C	T	T	T	T	chr1H	978839	978953
BOPA1_8670-388	G	G	G	C	C	C	C	chr1H	979555	979315
BOPA2_12_30944	C	C	C	A	A	A	C	chr1H	1913999	1914119
JHI-Hv50K-2016-1971	T	T	T	C	C	C	C	chr1H	1922371	1922491
JHI-Hv50K-2016-2146/QBS114	T	T	T	C	C	C	C	chr1H	2134052	2134166
JHI-Hv50K-2016-2374/QBS115	A	A	A	G	G	G	G	chr1H	2203522	2203642
JHI-Hv50K-2016-2914/QBD116	C	C	C	T	T	T	C	chr1H	2472669	2472789
JHI-Hv50K-2016-2931/QBS117	A	A	A	G	G	G	A	chr1H	2473584	2473704

2.6.2.2 Genotyping-by-sequencing (GBS)

Genomic DNA from same lines as for the 50K array was subjected to GBS genotyping. The normalized DNA (20 ng/µl) was cleaved, and quantified according to Wendler et al. (2014) with enzymes *Pst*I-HF (NEB R3140S) and *Msp*I (NEB R0106S). Sequencing was conducted on a MiSeq (Illumina, Inc., San Diego, CA) using the Illumina Kit V3 and 150 total paired-end cycles. Starting libraries consisted of genomic fragments with an average length of 434 bp which was determined with the High Sensitivity DNA Reagent on the Agilent Bioanalyzer, at an average concentration of 19.18 nM (Arora et al. 2019). Working libraries were diluted to an average DNA concentration of about 2 nM with EBT-Buffer (10 mM Tris-HCl pH 8.0; 0.1% Tween 20). The library was then

denatured and diluted to 10 pM in HT1 Puffer (Illumina sequencing kit, Illumina Inc. San Diego, CA) for the sequencing run on an Illumina MiSeq with the MiSeq Reagent Kit v3 (150-cycle) (MS-102-3001; Illumina Inc. San Diego, CA). The cluster density was1336 K/mm2. Across the MiSeq run, 86% of the clusters passed quality filtration, representing a matrix of multi-sequence alignment of about 4260.1 megabases (Mb) of the 5,100 Mb genome. Quality parameter Q30 reached 93.7%. Data were analysed using the Galaxy platform implemented at the JKI (Blankenberg et al. 2010, Giardine et al. 2005, Goecks et al. 2010). Trim Galore software from Babraham Bioinformatics (2012) (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) (trim galore version 0.2.8.1; quality <30, read length >50) was used for adapter and quality trimming of the amplified genomic sequences. After adapter and quality trimming, read mapping of the GBS data was conducted using BWA version 0.7.15-r1140 (Li and Durbin 2009) with standard settings to map the reads to the reference barley genome sequence "150831 barley pseudomolecules.fasta" (Mascher et al. 2017). SNP calling was done by means of the program sam tools mpileup version 1.2 (Li and Durbin 2009), with genotype likelihood computation. Imputation of missing data was implemented with the program Beagle v4.1 (Browning and Browning 2016). Once biallelic SNPs were detected, they were filtered for differences between the resistant and susceptible parental lines and a minimum coverage of five reads per SNP using the program SnpSift version 4.2 (Cingolani et al. 2012). Kompetitive Allele Specific PCR (Ramirez-Gonzalez et al.) primers were designed based on the polymorphic SNPs located in target region carrying $Rph_{MBR1012}$ (Table 5).

Table 5. GBS reads used for development of markers in the candidate interval.

Marker ID	Region
MBR1/QBS118	chr1H_[1923497:1923797]
MBR2/QBS119	chr1H_[2037511:2037894]
MBR3/QBS120	chr1H_[2112708 :2113233]
MBR4/QBS121	chr1H_[2118189 :2118624]
MBR5/QBS122	chr1H_[2128350 :2128692]
MBR6/QBS123	chr1H_[2145317:2145696]
MBR7/QBS124	chr1H_[2145621:2146005]
MBR8/QBS125	chr1H_[2205041:2205359]
MBR9/QBS126	chr1H_[2205938 :2206248]
MBR10/QBS127	chr1H_[2208847:2209147]
MBR11/QBS128	chr1H_[2239877:2240177]
MBR12/QBS129	chr1H_[2299778 :2300116]
MBR13/QBS130	chr1H_[2363016 :2363392]
MBR14/QBS131	chr1H_[2480277:2480664]
MBR15/QBS132	chr1H_[2534262 :2534562]

2.6.2.3 KASP markers analysis

Kompetitive Allele Specific PCR (KASP) was carried out in a RT-PCR thermal cycler (Tables 6 and 7). Two allele-specific primers, and one common primer were designed directly at the SNP position, using Primer3 version 4.1.0 (http://primer3.ut.ee/) (Koressaar and Remm 2007, Untergasser et al. 2012). Allele specific primers were marked with a FAM-Tail and the other one with a HEX-Tail. PCR amplifications were performed in 96 well plates (BioRad) in 5 µl reaction volume per sample containing 2.2 µl of template DNA (25 ng/µl), 2.5 µl of KASP-Reaction Mix (LGC Genomics GmbH, Germany), 0.08 µl forward primer, allele 1 (10.0 pmol/ µl, labeled with FAM M13 tail), 0.08 µl forward primer allele 2 (10.0 pmol/ µl, labeled with HEX M13 tail) and 0.2 µl reverse common primer (10.0 pmol/ µl). PCR was conducted on a BioRad PCR system using the following conditions: initial step at 94°C for 10 min, followed by 10 cycles at 94°C for 20 s, annealing at 61°C to 55°C (-0.6°C/cycle) for 60 s, followed by 26 cycles at 94°C for 20 s, 55°C for 60 s. After thermal cycling was complete, the fluorescent signal of FAM and HEX was detected by reading the plate in the qPCR machine (BIO-RAD CFX-series instruments) after incubation for 60s at 37°C. Automatic Allelic Discrimination allowed the identification of respective alleles (LGC, Guide to running KASP genotyping on the BIO-RAD CFX-series instruments).

All primer pairs first were tested on the two parental lines Scarlett and MBR1012 for functional efficiency and subsequent separation on a 1.5 % agarose gel. Primers with clear-cut polymorphism were mapped on the whole RIL population. During the mapping process, the RILs, which showed heterozygosity in many markers, were excluded from the genetic map.

Table 6. Primers of KASP-markers based on sequence information of 50K iSelect markers.

50K marker/primer	Sequence
JHI-Hv50K-2016-2146_com/QBS114	CCAACCGGTATCCTTAAAGTCA
JHI-Hv50K-2016-2146_T	GAAGGTGACCAAGTTCATGCTCCGTGTCCCGTACGCAT
JHI-Hv50K-2016-2146_C	GAAGGTCGGAGTCAACGGATTCGTGTCCCGTACGCAC
JHI-Hv50K-2016-2374_com/QBS115	GGGAGACCTTCAAGCTGTGG
JHI-Hv50K-2016-2374_A	GAAGGTGACCAAGTTCATGCTGAGGTTGAAGGTGTTCTGGTCA
JHI-Hv50K-2016-2374_G	GAAGGTCGGAGTCAACGGATTGGTTGAAGGTGTTCTGGTCG
JHI-Hv50K-2016-2914_com/QBS116	TGCTGGGCTGTATACTGAATGA
JHI-Hv50K-2016-2914_T	GAAGGTGACCAAGTTCATGCTCAGTTTTTGAACTGGAAAGTTTGTGTA
JHI-Hv50K-2016-2914_C	GAAGGTCGGAGTCAACGGATTGTTTTTGAACTGGAAAGTTTGTGTG
JHI-Hv50K-2016-2931_com/QBS117	CCATTAGCAGGATCACCATT
JHI-Hv50K-2016-2931_G	GAAGGTGACCAAGTTCATGCTACTTTGTACACGTTTGCTTATGTCC
JHI-Hv50K-2016-2931_A	GAAGGTCGGAGTCAACGGATTCTTACTTTGTACACGTTTGCTTATGTCT

Table 7. Sequence of markers originated from Genotyping by sequencing.

GBS Marker/primer	Sequence
MBR1_com/QBS118	GACCGCTCGTATACCCTTGA
MBR1_G	GAAGGTGACCAAGTTCATGCTCGCTGCAGTGTCGGGTC
MBR1_C	GAAGGTCGGAGTCAACGGATTCGCTGCAGTGTCGGGTG
MBR2_com/QBS119	TCGGCAACTAACCTGATTCC
MBR2_G	GAAGGTGACCAAGTTCATGCTCATGCATGTTTAGTTTCCAATCTC
MBR2_A	GAAGGTCGGAGTCAACGGATTCATGCATGTTTAGTTTCCAATCTT
MBR3_com/QBS120	GGTAGATCACGACGCAACC
MBR3_G	GAAGGTGACCAAGTTCATGCTCAGCGGCGGCTCTCG
MBR3_A	GAAGGTCGGAGTCAACGGATTCAGCGGCGGCTCTCA
MBR4_com/QBS121	AGTCCCACTGTCTTAGGGTC
MBR4_T	GAAGGTGACCAAGTTCATGCTTTTAGATAGACTGCAGATGGATATAGAGAA
MBR4_C	GAAGGTCGGAGTCAACGGATTTTTAGATAGACTGCAGATGGATATAGAGAG
MBR5_com/QBS122	GGCATATTGAGACCATGCAC
MBR5_G	GAAGGTGACCAAGTTCATGCTTTGCCTTACCTAGGATGCAAAG
MBR5_T	GAAGGTCGGAGTCAACGGATTTTGCCTTACCTAGGATGCAAAT
MBR6_com/QBS123	ACAAGCAATCACAAGCCAGT
MBR6_G	GAAGGTGACCAAGTTCATGCTCACTCTGCAGGTTCTGTTTCG
MBR6_A	GAAGGTCGGAGTCAACGGATTCACTCTGCAGGTTCTGTTTCA
MBR7_com/QBS124	GCAGGGTGGGTACAACAGAA
MBR7_A	GAAGGTGACCAAGTTCATGCTCATAAGCAGCCACCAAGTTATGAT
MBR7_C	GAAGGTCGGAGTCAACGGATTAAGCAGCCACCAAGTTATGAG
MBR8_com/QBS125	AGTTTGAAGGTCTCCCAGCT
MBR8_C	GAAGGTCGGAGTCAACGGATTCAGTTCAGGTCCGTGACG
MBR9_com/QBS126	TGGTTGATCCGATAGAGCTTG
MBR9_G	GAAGGTGACCAAGTTCATGCTATCGCCAACCGTCCCTC
MBR9_A	GAAGGTCGGAGTCAACGGATTATCGCCAACCGTCCCTT
MBR10_com/QBS127	CCCTATGGCCAATTCATTTC
MBR10_T	GAAGGTGACCAAGTTCATGCTCCACCCAAAACCTACAAGCA
MBR10_C	GAAGGTCGGAGTCAACGGATTCCACCCAAAACCTACAAGCG
MBR11_com/QBS128	ACGAGCGCACTGCAGAATTA
MBR11_T	GAAGGTGACCAAGTTCATGCTGCTCACACCATTGTCTTCTTCTTT
MBR11_C	GAAGGTCGGAGTCAACGGATTGCTCACACCATTGTCTTCTTCTTC
MBR12_com/QBS129	TGGCACGATACCGAAACC
MBR12_G	GAAGGTGACCAAGTTCATGCTGAACCCAAGGGTCCATTTTC
MBR12_A	GAAGGTCGGAGTCAACGGATTGAACCCAAGGGTCCATTTTT
MBR13_com/QBS130	GAGAGGTGCAGGCAGACG
MBR13_A	GAAGGTGACCAAGTTCATGCTCTGACATGTTGGGTGGGACT
MBR13_T	GAAGGTCGGAGTCAACGGATTTGACATGTTGGGTGGGACA
MBR14_com/QBS131	CACTCATCCACGCAGCAC
MBR14_G	GAAGGTGACCAAGTTCATGCTCCCATGTTAGACGCGACG
MBR14_C	GAAGGTCGGAGTCAACGGATTTCCCATGTTAGACGCGACC
MBR15_com/QBS132	CTTCGAACATGCCTCCAAAC
MBR15_C	GAAGGTGACCAAGTTCATGCTGAGGCTATAACTTGTTGTGAAACATTC
MBR15_A	GAAGGTCGGAGTCAACGGATTGAGGCTATAACTTGTTGTGAAACATTA

2.7 Linkage analysis

Linkage analysis was performed by dividing the number of the recombination events by the number of analysed gametes, multiplied with 100, giving percentage of recombinations. The recombination frequency was used for the genetic linkage map construction and visualized using MapChart (Voorrips 2002) software package.

$$\text{Recombination frequency} = \frac{\text{Number of the recombination events}}{\text{Total number of analysed gametes}} \times 100$$

2.8 Testing the diagnostic value of newly developed markers

In order to get information on the diagnostic value of co-segregating markers, a set of 25 barley genotypes/lines carrying *Rph1* to *Rph25* resistance genes (see table 8), 23 parental lines as well as 15 introgression Bowman lines carrying *Rph1* to *Rph15* were chosen (Table 8). Leaves of 14 days old selected plants were used for DNA extraction according to (Stein et al. 2001). $Rph_{MBR1012}$ co-segregating markers i.e. QBS128, QBS116, QBS117, GBS626, GBS534 and GBS546, as well as some markers in the vicinity to the $Rph_{MBR1012}$ locus i.e. GBS564, GBMS187, QBS98, QBS2 and QBScg134, were evaluated on selected barley genotypes/lines. The diagnostic value of tested co-segregating markers was calculated using the following equation:

$$\text{Diagnostic value} = \frac{\text{Number of lines showing different allele of MBR1012}}{\text{Total number of analysed lines}} \times 100$$

Table 8. Selected Bowman lines and parental lines carrying 25 known *Rph* genes for diagnostic value evaluation of developed markers linked to the resistance locus.

Cultivar/lines	*Rph*-Gene	Gene Locus	Locus
MBR1012	Resistance/$Rph_{MBR1012}$	1HS	(König et al. 2012)
Scarlett	*Rph3/Rph9/Rph12*	7HL/5HS/5HL	(Jin et al. 1993, Borovkova et al. 1998)
Oderbruker	*Rph1*	2H	(Tuleen and McDaniel 1971, Tan 1978)
B.L.195-246-1	*Rph1*	2H	(Roane and Starling 1967)
Peruvian	*Rph2*	5HS	(Borovkova et al. 1997, Franckowiak et al. 1997)
B.L.195-266-1	*Rph2*	5HS	(Borovkova et al. 1997)
B.L.193-343-1	*Rph2*	5HS	(Borovkova et al. 1997)
Estate	*Rph3*	7HL	(Jin et al. 1993)
B.L.195-267-2	*Rph3*	7HL	(Jin et al. 1993)
Gold	*Rph4*	1HS	(McDaniel and Hathcock 1969)
B.L.195-268-4	*Rph4*	1HS	(McDaniel and Hathcock 1969)
Magnif	*Rph 5*	3HS	(Mammadov et al. 2003)
B.L.195-269-1	*Rph5*	3HS	(Mammadov et al. 2003)
Bolivia	*Rph2+6*	5HS+3HS	(Zhong et al. 2003)
B.L.195-270-2	*Rph6*	3HS	(Brunner et al. 2000)
Cebad capa	*Rph 7*	3HS	(Brunner et al. 2000, Graner et al. 2000)
B.L.193-21	*Rph7*	3HS	(Brunner et al. 2000)
B.L.196-424-1	*Rph7*	3HS	(Brunner et al. 2000)
Egypt4	*Rph8*	7HS	(Borovkova et al. 1997)
B.L.195-349-4	*Rph.8h*	7HS	(Borovkova et al. 1997)
Trumph	*Rph9+12*	5HL	(Borovkova et al. 1998)
B.L.194-224	*Rph9*	5HS	(Borovkova et al. 1998)
B.L.195-274-1	*Rph9*	5HS	(Borovkova et al. 1998)
BC8	*Rph 10*	3HL	(Feuerstein et al. 1990)
B.L.195-272-1	*Rph10*	3HL	(Feuerstein et al. 1990)
BC67	*Rph 11*	6HS	(Feuerstein et al. 1990)
B.L.195-273-2	*Rph11*	6HS	(Feuerstein et al. 1990)
195-288-2	*Rph13*	7HS	(Sun and Neate 2007)
195-290-2	*Rph14*	7HS	(Golegaonkar et al. 2009a)
B.L.195-282-2	*Rph15*	2HS	(Weerasena et al. 2004)
Hordeum spontaneum 680	*Rph16*	2HS	(Ivandic et al. 1998)
NGB22914	*Rph 17*	2HS	(Pickering et al. 1998)
NGB22900	*Rph 18*	2HL	(Pickering et al. 2000)
Prior	*Rph 19*	7HL	(Park and Karakousis 2002)
Flagship	*Rph 20*	6H	(Hickey et al. 2011)
Ricardo	*Rph 21*	4H	(Sandhu et al. 2012)
NGB22893	*Rph 22*	2HL	(Johnston et al. 2013)
Yerong	*Rph 23*	7HS	(Singh et al. 2015)
ND24260-1	*Rph 24*	5HS	(Ziems et al. 2017)
Fongtien	*Rph 25*	5HL	(Kavanagh et al. 2017)
Reka1	*Rph3+?*	7HL+?	(Jin et al. 1993)
HOR4280	*Rph1d+1r*	2H	(Roane and Starling 1967)

Table 8. continued

Cultivar/lines	*Rph*-Gene	Gene Locus	Locus
Bowman	*Susceptible*	-	-
Bowman	*Rph15*	2HS	(Weerasena et al. 2004)
HOR500-1	*Rph1d+1r*	2H	(Roane and Starling 1967)
Grossklappige	*Susceptible*	-	-
Sudan	*Rph1*	2H	(Roane and Starling 1967)
Quinn	*Rph2+5*	5HS+3HS	(Borovkova et al. 1997, Mammadov et al. 2003)
Rika × F_1	*Rph3*	7HL	(Jin et al. 1993)
Lada	*Susceptible*	-	-
Krona	*Rph12*	5HL	(Borovkova et al. 1998)
Alexis	*Susceptible*	-	-
HOR679-3	*Rph3*	7HL	(Jin et al. 1993)
Vada	*Partial res.*	-	-
HOR1132	*Rph2r*	5HS	(Borovkova et al. 1997)
HOR1063	*Partial res.*	-	-
Salome	*Susceptible*	-	-
HOR2596	*Rph9*	5HS	(Borovkova et al. 1998)
Emir	*Susceptible*	-	-
Karat	*Susceptible*	-	-
L94	*Susceptible*	-	-
MBR532	*Susceptible*	-	-
Igri	*Susceptible*	-	-

2.9 Anchoring *Rph*$_{MBR1012}$ fine map to the barley reference genome sequence

A FASTA file of the sequences of all 56 markers mapped at the high resolution mapping population (HRMP) including forward and revers primers was blasted against the barley reference genome sequence (http://webblast.ipk-gatersleben.de/barley_ibsc/) using BLASTN algorithm applying default parameters. Obtained physical positions of mapped markers were visualized using the software MapChart (Voorrips 2002). The interval between flanking markers at the barley reference sequence was used to extract the putative candidate genes (https://plants.ensembl.org/Hordeum_vulgare/Info/Index). The High-Confidence and Low-Confidence genes in the narrowed target interval and the exon/intron boundaries of five identified disease resistance genes in the target interval were extracted from available annotation (Mascher et al. 2017). The reconstruction of the gene intron-exon-structure was performed by using 'Splign' (https://www.ncbi.nlm.nih.gov/sutils/splign/splign.cgi?textpage=online&level=form) from NCBI, which allows alignment of mRNA to genomic sequence (Kapustin et al. 2008).

2.10 Allele specific re-sequencing of candidate genes

To reduce the number of defined candidate genes in the target interval comprising 18 high and 11 low confidence genes, allele specific re-sequencing was conducted. All primers were designed using online software Primer3 v. 0.4.0 (http://bioinfo.ut.ee/primer3-0.4.0/) (Koressaar and Remm 2007, Untergasser et al. 2012), setting the parameters at 20-22bp, temp. 58-62°C and product size of 350 bp. Synthetized primers were tested for their specificity for chromosome 1H using barley blast server (https://webblast.ipk-gatersleben.de/barley_ibsc/) against the barley pseudomolecules (Mascher et al. 2017). In the first round of low pass resequencing, a set of 36 primer pairs covering all 29 high and low confidence genes in the target interval was designed (see appendix 9). In the second round of the experiment, a set of 25 primer pairs covering only five disease resistance genes was designed (see appendix 11). In the third round, 24 primer pairs were designed covering the full sequence length of five disease resistance genes in the target interval (Appendix 12). To sequence the disease resistance candidate genes in a second and third run of primer design, the Morex contigs including the gene sequence of each disease resistance gene were identified using (https://webblast.ipk-gatersleben.de/barley_ibsc/) allowing to design primers at least 20 bases upstream of the start codon and 20 bases downstream of the stop codon. Moreover, the primers overlapped each other to ensure that there are no gaps between the fragments after sequence analysis. A fragment size of 400 bp to 1,200 bp was chosen because of the maximum sequencing length. Amplification was done on the parental genotypes MBR1012 and Scarlett, as well as two DH lines (DH3/62, DH3/127), and "Morex" (as a control of the reference barley genome). Amplification reaction was prepared in a total volume of 20 µl (see Appendix 1). Two microliters of PCR product mixed with 8 µl 1X loading dye was loaded on a 1 % agarose gel and separated electrophoretically at 95 V for 2 h - 3 h in 1X TBE-buffer and analysed using the imaging system Gel Doc™ XR and the Quantity One® 1-D analysis software (4.6.2) (Bio-Rad, Hercules, USA). For sequencing, two aliquots of each PCR-product were transferred in special plate and added with 1 µl of the forward and reverse primer (10 pmol μl^{-1}), respectively. The aliquots were sent for sequencing to Microsynth AG (Balgach, Switzerland) using the Sanger sequencing method (Sanger et al. 1977). Those primers which presented a clear-cut polymorphism between MBR1012 and Scarlett were mapped in the developed RILs population. Sequencing data was analysed using Sequencher 5.1 software (Gene Codes, Ann Arbor, MI) using default parameters. Functional analysis of identified polymorphisms between parental lines (MBR1012 and Scarlett) was done using the multiple sequence alignment program, MAFFT by default parameters (Katoh and Standley 2013).

3 Results

3.1 High-resolution genetic mapping and marker saturation of the leaf rust resistance gene *Rph$_{MBR1012}$*

Rph$_{MBR1012}$ conferring barley leaf rust resistance was genetically mapped between the two markers MBR546 and GMS021 in the telomeric region of chromosome 1H in an F_1-derived DH population of 91 individuals (König et al. 2012). A high resolution mapping population was constructed using four crosses i.e. DH 3/74 (S) × DH3/6 (R), DH3/74 (S) × DH3/127 (R) , DH3/6 (R) × DH3/9 (S) and DH3/62 (S) × DH3/127 (R) involving two subsequent steps (Table 9). In a first step, 2,175 F_2-plants providing a genetic resolution of 0.23 % recombination were screened for the recombination between the flanking markers QBS94 (distal) and QBS113 (proximal) and a genetic distance of 8.0 cM was determined between QBS94 and QBS113. In second round, additional 2,663 F_2 plants were screened giving resolution of 0.02 % recombination. Finally, in total out of a set of 5237 analysed F_2 plants 4775 survived. From corresponding F_3 families 537 recombinant F_4 RILs were developed. During the mapping process, the RILs, which turned out to be heterozygous were excluded from the genetic map. In first marker saturation thirty-seven primer pairs were designed including 23 genome zipper markers and 14, 9K iSelect SNP markers (Appendix 8) and were integrated into the existing map based on 537 segmental RILs. Consequently, first marker enrichment resulted in shortening the target interval to 0.1 cM (Figure 6.

Table 9. DH lines used for crossing and high resolution mapping population construction.

Crosses	Number of analysed F_2	Selected 537 homozygous segmental RILs (F_4)		$\chi 2$ (df = 1, p < 0.05)
		Resistant	Susceptible	
DH3/74 (S) x DH3/6 (R)	389	32	29	0.1475
DH3/74 (S) x DH3/127 (R)	1469	88	72	1.6
DH3/6 (R) x DH3/9 (S)	713	45	53	0.653
DH3/62 (S) x DH3/127 (R)	2204	96	122	3.1009
Total	4775	261	276	0.4189

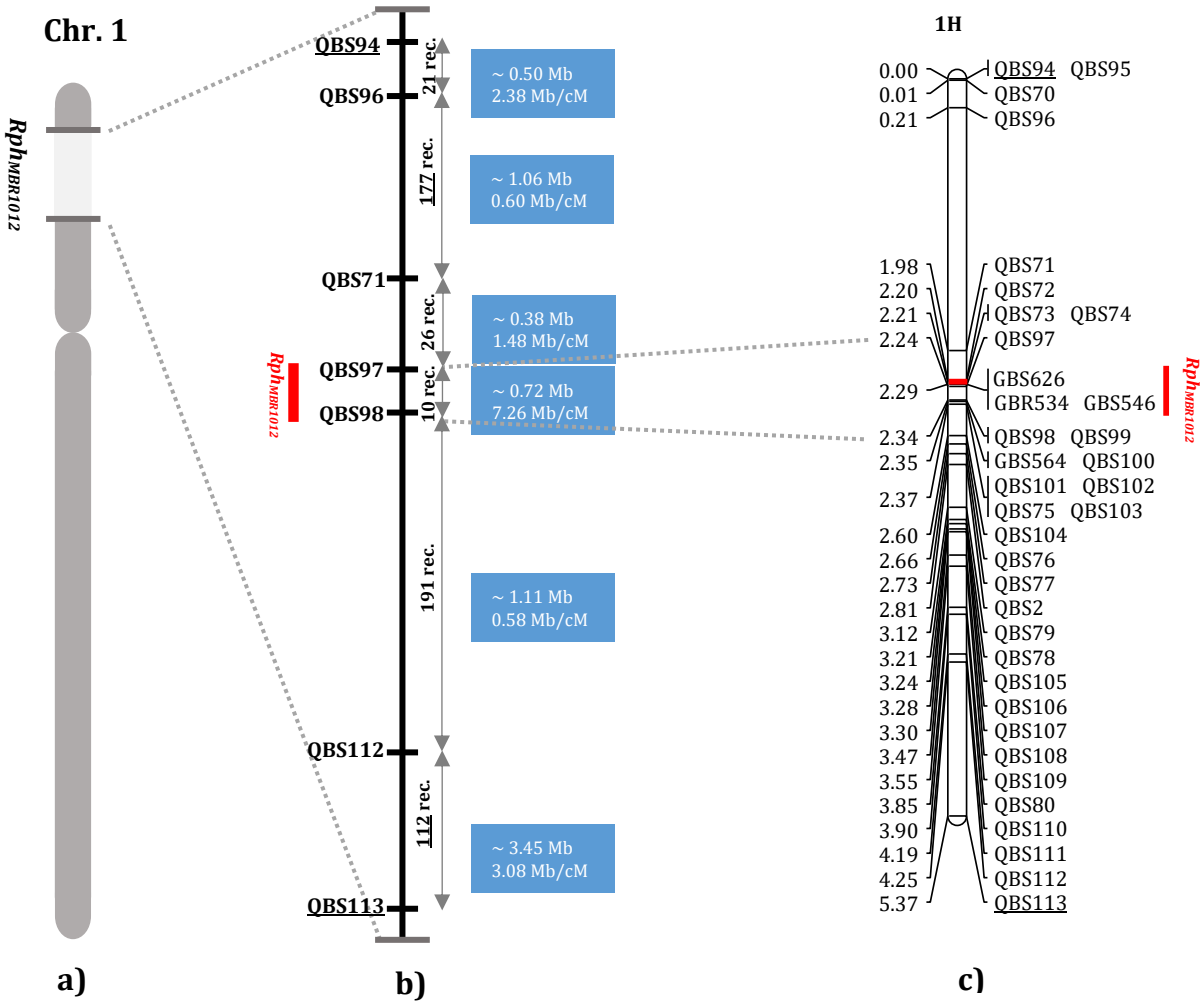

Figure 6. High-resolution genetic map of *Rph*$_{MBR1012}$. a) The genomic region harbouring *Rph*$_{MBR1012}$. b) The identification of 537 recombinants and mapping of *Rph*$_{MBR1012}$ locus based on markers derived from the Genome Zipper and the 9K iSelect chip flanked by QBS97 and QBS98. The blue boxes indicate the physical size based on the reference sequence. c) Genetic map of the *Rph*$_{MBR1012}$ locus.

3.1.1 50K iSelect genotyping

The 50K screen revealed in total a set of 40,777 scoreable SNPs. Out of these, 14,616 SNPs showed homozygous polymorphisms between resistant and susceptible genotypes (Figure 7). Thirty-nine SNPs were located at the large interval of 8.0 cM on chromosome 1HS, and four SNPs were located within the target interval comprising 0.1 cM (see table 4). These SNPs were converted into KASP markers and mapped on the whole HRMP population.

3.1.2 Genotyping-by-sequencing (GBS)

Genotyping by sequencing analysis yielded 48,226 SNPs distributed over all seven barley chromosome, of which 37,287 showed homozygous polymorphisms between resistant and susceptible lines (Figure 7). Out of these, 80 polymorphic markers were located in the larger interval (8.0 cM) and 15 SNPs were identified in the shortened interval of 0.1 cM (see table 7). KASP markers were designed for all 15 SNPs and used for genotyping of the 537 RILs.

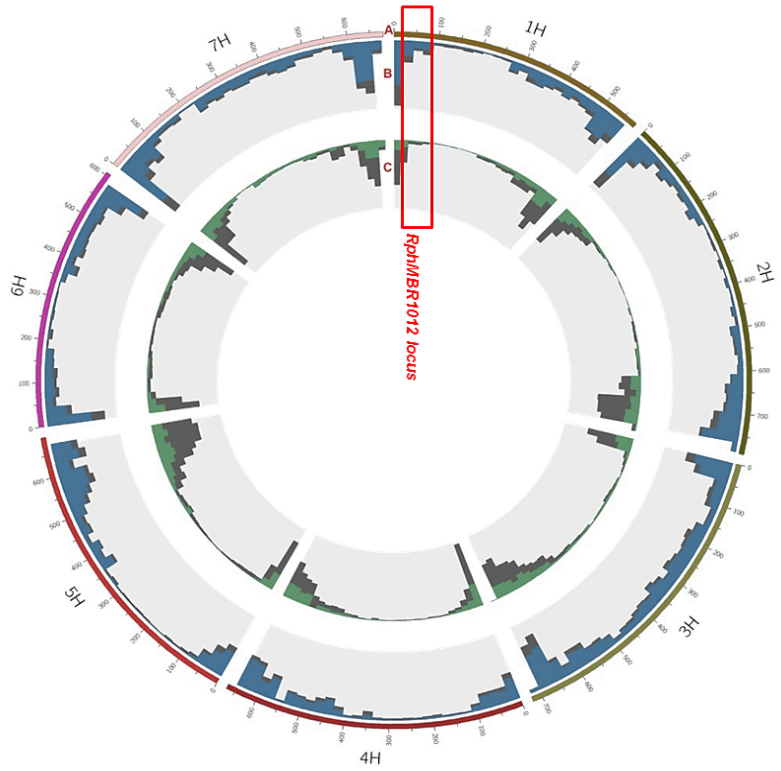

Figure 7. Genome-wide distribution of 50K and GBS SNPs. Track A: Gives the seven barley chromosomes. Track B: Grey colour depicts MBR: (all 48,226), Blue: position of MBR1012= Scarlett homozygous markers; polymorph: 37,287. Track C: Distribution of SNP Chip (50K): Grey (all 40,777), Green: position of MBR1012 = Scarlett homozygous markers; polymorph: 14,616.

Finally, 56 primer pairs were mapped in the target region on chromosome 1HS. Primers, QBS71, QBS106, QBS98 and QBS99 are size polymorphic markers while QBS96,QBS78 and QBS110 were dominant markers ABI Genetic Analyser was used for markers GBR534, GBMS187, GBS564, QBS2, QBS94 and QBS113 since the difference between to the parental lines was less than 2 bp, which could not be detected on 1.5% agarose gel. Tables 2-3 and 6-7 gives a detailed overview about all markers that have been developed and used for mapping. High-resolution mapping allowed us to increase the map resolution from 0.023% recombination in to 0.010% recombination As a result;

the *Rph*$_{MBR1012}$ gene was positioned between markers QBS127 on the distal side and QBS98 on the proximal side. Hence, the distal and proximal border of *Rph*$_{MBR1012}$, was defined by seven recombination events between QBS127 and QBS98. The target interval decreased from 8.0 cM to 0.07 cM as well. Moreover, it turned out that distribution of recombination within the target interval was uneven varying from 0.58 Mb/cM and 0.60 Mb/cM proximally and distally, respectively to 7.26 Mb/cM at the *Rph*$_{MBR1012}$ locus (see figure 6). Marker saturation also revealed a high number of recombinations, i.e. 177 and 112 recombinations between markers located up and down-stream of the target interval (Figure 8). Ten molecular markers i.e. QBS128, QBS129, QBS130, GBS626, GBR534, GBS546, QBS116, QBS117, QBS131 and QBS132 were co-segregating with the *Rph*$_{MBR1012}$. Therefore, these ten new markers may be the markers of choice as candidate markers closely linked to the resistance locus.

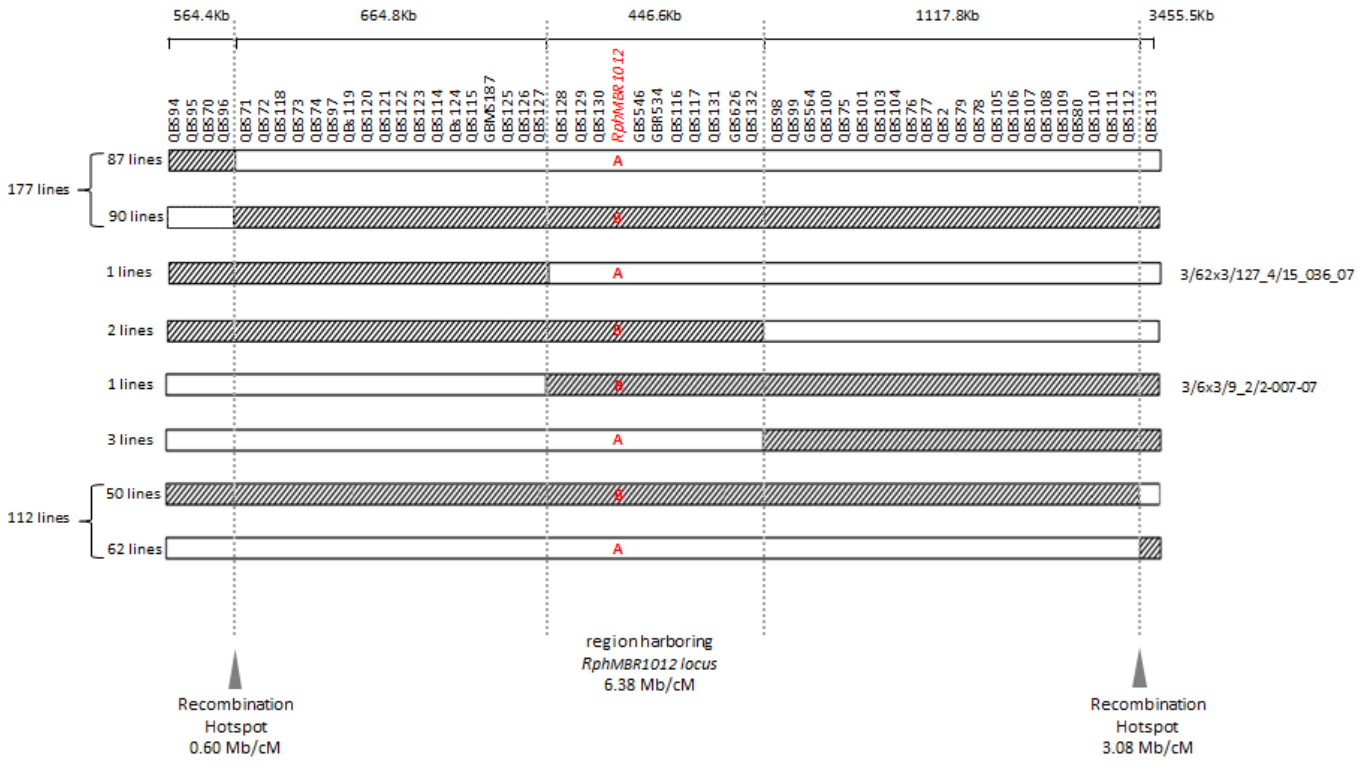

Figure 8. Graphical genotypes of F_4 RILs for all 537 recombinant lines carrying cross-over events between QBS94 and QBS113 (8.0 CM). A (Susceptible genotype = white) and B (Resistant genotype = hatched) in the target locus indicate the result of the resistance test of recombinant lines. Border of hatched to white shows the recombination position between the MBR1012 allele to the Scarlett allele and white to hatched shows the recombination position between the Scarlett allele to the MBR1012 allele.

3.2 Phenotyping of homozygous recombinants inbreed lines (RILs) in the *Rph*$_{MBR1012}$ region

3.2.1 Macroscopic assessment

Phenotypic analysis of resistance to *Rph*$_{MBR1012}$ showed a segregation of 261 resistant and 276 susceptible RILs and revealed the expected 1r:1s segregation ratio among these RILs. Chi-square test (X^2 1:1 = 0.4189, df = 1, $p < 0.05$) for goodness of fit indicated that the resistance in MBR1012 is monogenically controlled (Figure 9 and Table 9).

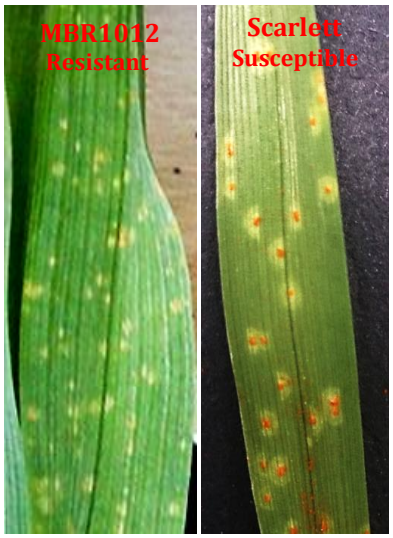

Figure 9. Disease symptoms on resistance (MBR1012) and susceptible parent (Scarlett).

3.2.2 Microscopic investigation

Employment of three different staining methods, including Calcofluor White M2R, 3,3'-diaminobenzidine (DAB) and Propidium Iodide, suggested that leaf rust defence is hypersensitive response (HR)-mediated. Evaluation of fungal infection structures s was possible using Calcofluor white M2R staining. Spore germination, germ tube and appressorium formation was observed within 18 h after inoculation. After the appressorium formation, other infection structures including infection peg, sub-stomatal vesicle, infection hyphae and haustorial mother cells (HMC) were observed at 24 h post inoculation (hpi) (Figure 10). At later time points (48, 72 and 96 hpi), an increased number of HMC and further colony development was observed. At seven days post inoculation (dpi) completely established colonies with sporogenic tissue was visible in the susceptible genotype Scarlett (Figure 10). In Scarlett, matured and newly formed urediniospores were detected within the established colonies by 8 dpi. The evaluation of intercellular hyphal

growth showed that the development of hyphae was suppressed at the HMC stage in the resistant genotype MBR1012 and the F_1 at 24 hpi due to HR. However, in the susceptible genotype Scarlett no defence reaction was detectable. In Calcofluor white M2R staining, the HR was identified by brownish colour of plant mesophyll cells, which is due to the accumulation of phenolic compounds. Fluorescence microscopy showed that in both resistant and susceptible leaves the fungus is able to establish infection structures, but cannot complete its life cycle and produce urediniospore in resistant genotypes. These results confirm that the fungal infection is stopped at an early stage of the infection process (Figure 10, F).

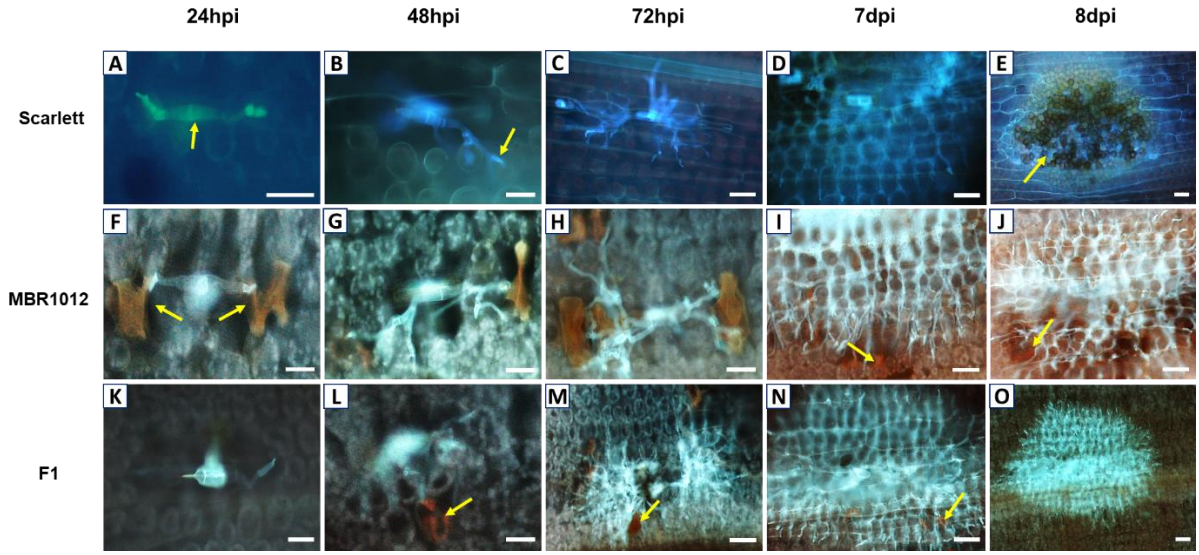

Figure 10. Development of fungal structures in the susceptible cultivar Scarlett (A–E) the resistant cultivar MBR1012 (F–J) and F_1 (K-O). Microscopic observations of 24 hpi (A,F,K), 48 hpi (B,G,L). 72 hpi (C,H,M), 7 dpi (D,I,N), and 8 dpi (E,J,O) are compared. Arrows in (A) mark the substomatal vesicle, in (B) haustorial mother cells, in (E) urediniospores in successful infection, and in (F,I,J,L,M,N) hypersensitive reaction indicated by dark brown precipitate. Bars in (A,C,D,E,I,J,K,M,N,O): 20 µm and in (B,F,G,H,L): 10 µm.

In addition, two other well-known staining methods, i.e. 3,3'-diaminobenzidine (DAB) and Propidium Iodide staining were employed in order to evidently confirm our observation with Calcofluor white M2R staining regarding the HR. In both staining methods, normal light microscopy was used. DAB is oxidized by hydrogen peroxide (H_2O_2), to generate a dark brown precipitate. Hydrogen peroxide (H_2O_2) accumulates at the attack site during an incompatible interaction in resistant genotypes resulting in an oxidative burst. Our observations in MBR1012 confirm this phenomenon giving also a hint to a HR response (Figure 11). No precipitation was observed in Scarlett due to lack of defence responses as expected for a compatible interaction.

Propidium Iodide staining is mainly used to determine the cell viability during infection. Propidium iodide passes through damaged cell membranes and intercalates with DNA resulting in a bright orange-red colour explicitly in dead cells. In our study, the orange-red colour only was observed in the resistant genotype MBR1012 (Figure 11).

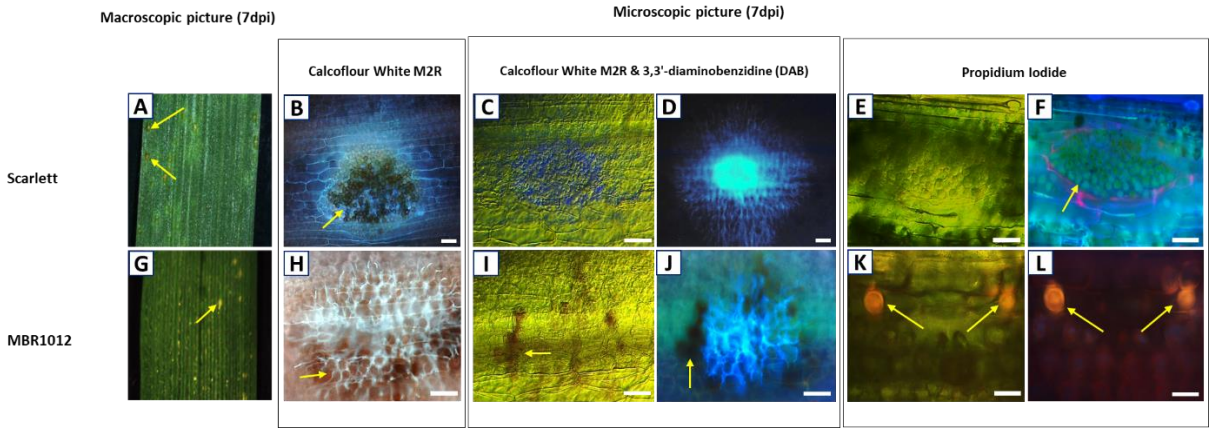

Figure 11. Macroscopic and microscopic symptoms caused by *Puccinia hordei,* isolate (I-80) on parental lines, MBR1012 and Scarlett seven days post inoculation. Macroscopic observation (A,G) and microscopic observation (B-F and H-L). Three different staining methods including: Calcofluor White M2R (B,H), 3,3'-diaminobenzidine (C,D,I,J), and Propidium Iodide (E,F,K,L) are compared. Arrows in (A) necrosis, in (B,F) urediniospores in successful infection, in (G) chlorosis, in (H,I,J,K,L) hypersensitive reaction indicated by dark brown and bright orange-red precipitate. Bars in (B,C,D,E,F,H,I,J): 20 µm and in (K,L): 10 µm.

3.3 Physical mapping and anchoring leaf rust resistance locus to the physical map of barley

In a BLAST search, almost all markers showed significant hits to the barley reference genome (Figure 12). Nearly perfect collinearity in our target interval was observed, with the exception of the distal part of the target interval comprising 1.34 Mb (15 markers). The physical size of the large target interval comprising 8.0 cM between flanking markers QBS94 and QBS113 comprises 6.24 Mb. Overall, 299 genes, i.e. 183 high confident and 116 low confident (LC) genes were detected. Based on the sequence annotation of HC and LC genes, 23 genes turned out to be disease resistance proteins and three were annotated as powdery mildew resistance proteins. The narrowed down region (QBS127 and QBS98) was estimated at 0.44 Mb comprising 11 low-confident and 18 high-confident genes. Fifteen of these genes are functionally annotated and five

of them are related to pathogen resistance. Mapping of additional two polymorphic markers originating from genes in the target interval reduced the physical size of the locus carrying $Rph_{MBR1012}$ to 0.350 Mb, between QBS127 and QBScg134. Within the narrowed collinear regions between markers QBS127 and QBScg134, a total number of 23 genes including 8 low-confidence and 15 high-confidence genes was located (Figure 12). Out of 23 genes identified in this interval, five genes were related to pathogen resistance, i.e. HORVU1Hr1G000830 (disease resistance protein), HORVU1Hr1G000840 (powdery mildew resistance protein PM3 variant), HORVU1Hr1G000860 (disease resistance protein), HORVU1Hr1G000900 (disease resistance protein) and HORVU1Hr1G000910 (disease resistance protein). Moreover, all five putative disease resistance genes carry conserved sequence motifs of Nucleotide-binding site (NBS) and leucine-rich repeats (LRR).

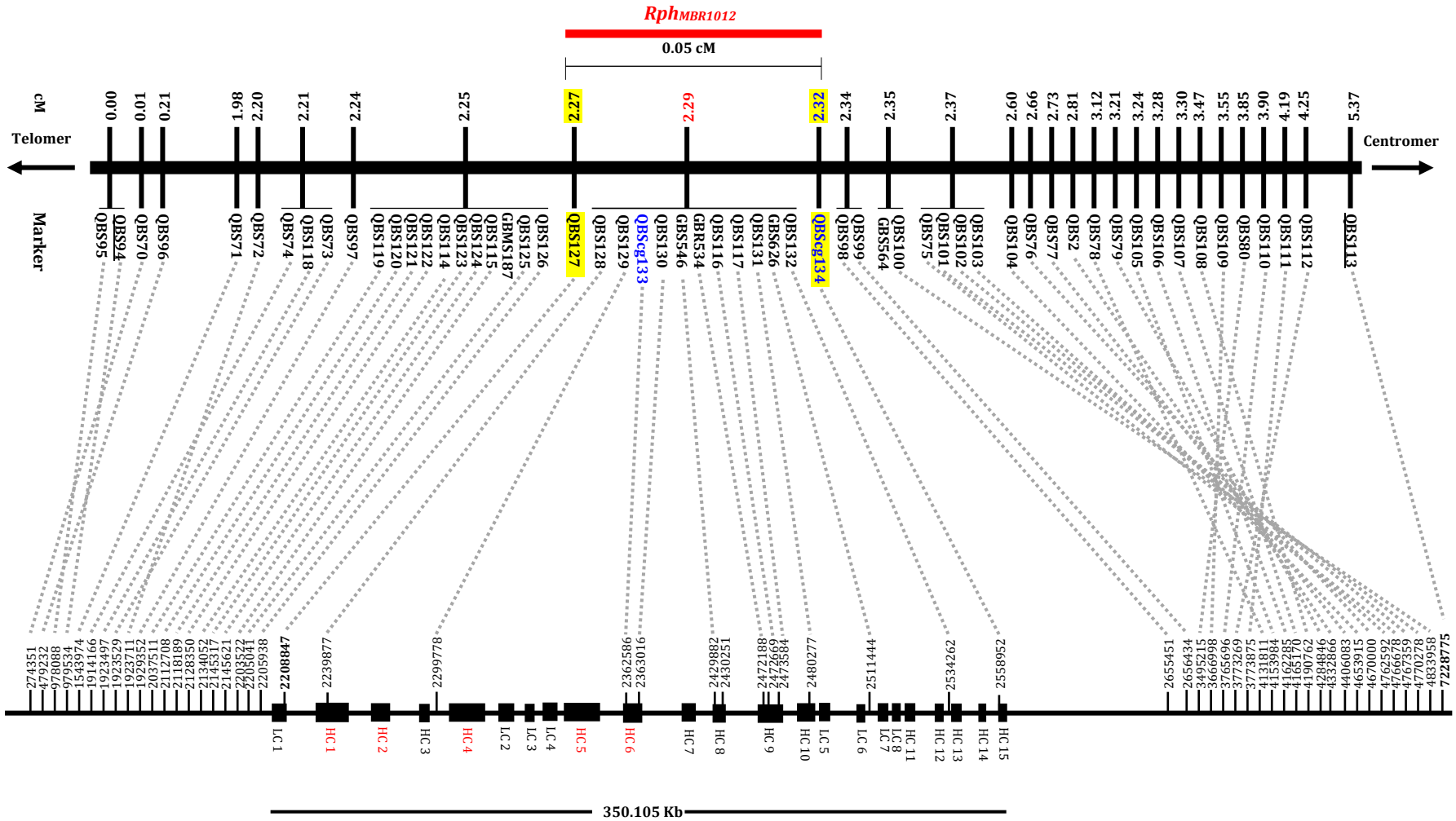

Figure 12. Co-linearity between genetic positions of mapped markers and their linear order based on the barley reference genome. Especially, lower part of target interval shows a deviation between genetic order and the corresponding order of the reference genome. All markers in target interval exhibited a good collinearity between genetic map and barley reference genome. Flanking markers were highlighted in yellow, mapped markers from candidate genes are blue and disease resistance genes are indicated by red color.

Annotation of the five disease resistance genes in target interval revealed an intron exon structure for HORVU1Hr1G000830 and HORVU1Hr1G000860 while HORVU1Hr1G000840, HORVU1Hr1G000900 and HORVU1Hr1G000910 consist of only one coding exon (Figure 13 and 14).

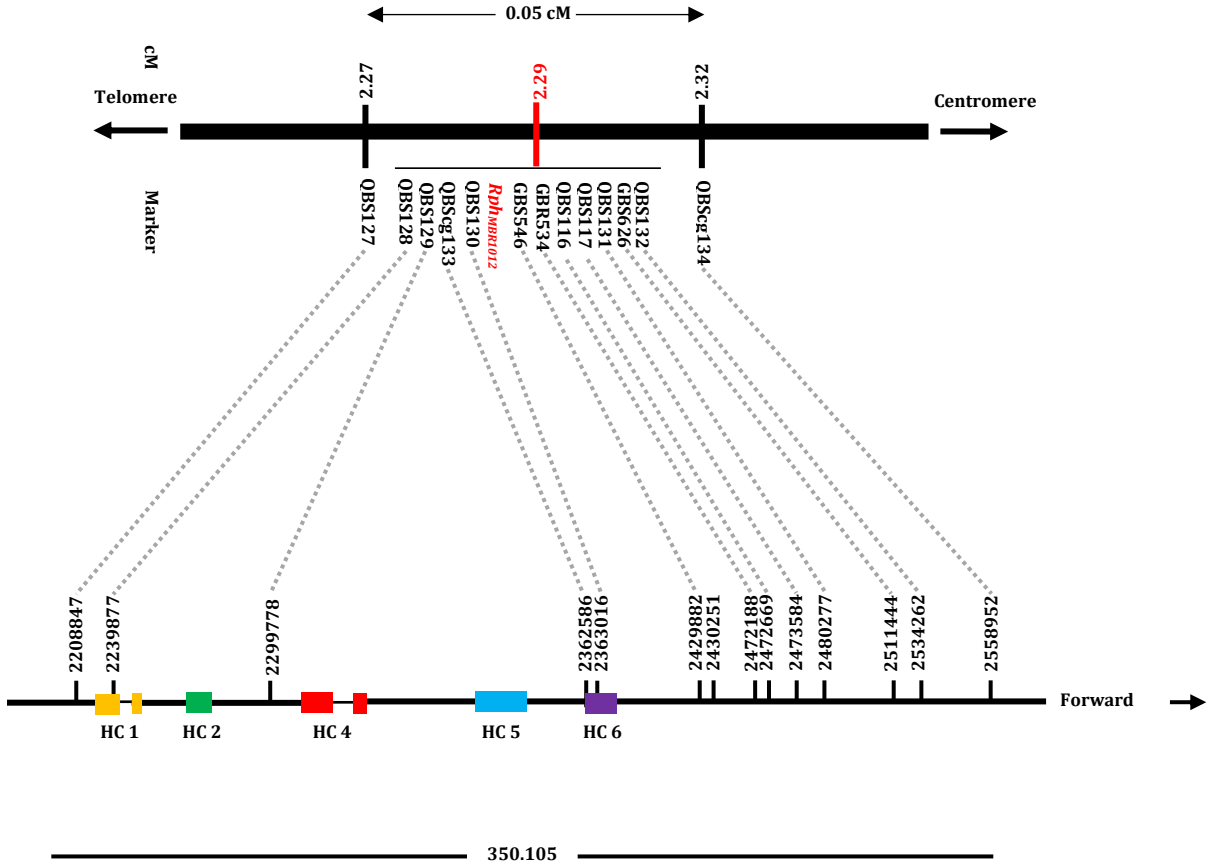

Figure 13. Gene structure of five putative disease resistance genes located in target interval harbouring *Rph*$_{MBR1012}$ delimited by markers QBS127 and QBS98. Coloured boxes represent gene exons and narrow lines shows intron.

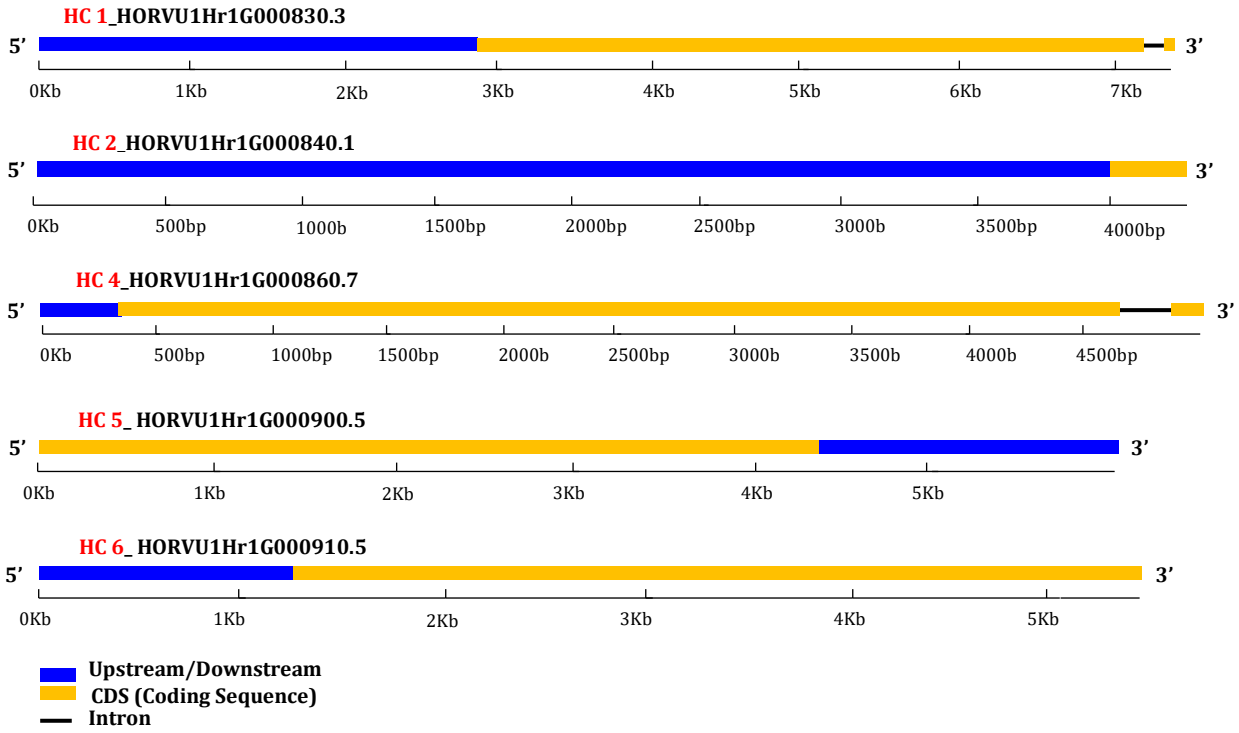

Figure 14. Results of the annotation of the disease resistance genes located in target interval of 0.05 cM.

3.4 Allele specific re-sequencing of candidate genes from the interval

Allele specific re-sequencing for all 29 putative genes located on the pseudomolecule of chromosome 1H (chr1H: 2,206,515 to 2,763,382) located in the narrowed interval comprising 0.44 Mb was conducted. In the first round of primer design, a set of 36 primer pair combinations was designed for low pass resequencing covering all 29 high and low confidence candidate genes (see Appendix 9). For two genes no specific primer on chr1H was developed due to the high similarity of the sequence of these genes (e.g. HORVU1Hr1G000820.1: on chromosome 4H, 1863 bp of 1866 bp identical to chromosome 1H). Among 36 primer pairs, three primers; two for MBR1012 (HORVU1Hr1G000830.3_s3635_as3910/290bp), (HORVU1Hr1G000840.1_s51_as204/270bp) and one for Scarlett (HORVU1Hr1G001000.1_s4_as158/290bp) showed a dominant pattern while all other 33 primers produced fragments in both parental lines. Additionally, two primer pairs (HORVU1Hr1G000910.9_s3958_as4143/QBScg133) and (HORVU1Hr1G001060.1_s173_as480/QBScg134) showed a direct size polymorphism on a 1.5 % agarose gel (Figure 15) and were subsequently mapped into the complete set of 537 RILs. Finally, 24 PCR amplicons of the primer pairs was functional while 12 primer pairs were not functional, i.e. PCR products gave multiple bands, smear or present/absent patterns. Finally, 24 PCR

amplicons of the functional primer pairs were sequenced (Appendix 10). Editing of allelic specific sequences revealed that a set of 18 primer pairs resulted in sequence information that could be perfectly aligned in both parental lines, while for six fragments no alignment could be obtained, either due to the low quality of the sequence data or due to heterozygous signals (Table10).

In a second round, we focused on the design of 25 primer pairs from five disease resistance genes (Appendix 11). 23 primer pairs amplified products in both parental lines. One was dominant by amplifying products in Scarlett and one was dominant for MBR1012 (morex_contig_51837_s6788_as7743/940bp) and did not produce any fragment in Scarlett (morex_contig_51837_s5063_as6187/1125bp). In total 12 PCR amplicons, which showed a clear single band for both MBR1012 and Scarlett were subjected to sequencing (Appendix 12). In a third round for whole length amplification and re-sequencing of five disease resistance genes in the target interval, 24 primer pairs were designed (Appendix 13). Among them, 18 primers amplified products in both parental lines. Five were genotyped based on the presence or absence of the PCR amplicon in one of the parental line: 3 for Scarlett (HORVU830.3_s6716/morex_contig_127934_as3989, morex_contig_51837_s5063_as5675, HORVU840.1_s4_as312) and 2 for MBR1012 (HORVU830.3_s2648_as3379, m_c_54254_s7176_as8025). At the same time, three markers were mapped as a size polymorphic ones (HORVU830.3_s5361_as6344, m_c_51837_s5568_as6270, m_c_51837_s2848_as3729). From this experiment, 12 PCR products were sequenced.

Finally, for 31,204 bp of all 29 candidate genes 85 primers were designed resulted in sequence information of about 17,107 bp in MBR1012 and 16,963 bp in Scarlett. 259 SNPs were identified for disease resistance genes from the target interval. Results of 61 primer pairs is presented in Table 10. Moreover, in gene HORVU1Hr1G000900.5 (Disease resistance protein) a large deletion (InDel) was identified in Scarlett ranging from 26 bp to 222 bp in comparison to MBR1012. Seven SNPs for HORVU1Hr1G000830.3, nine for HORVU1Hr1G000860.7 and 243 SNPs for HORVU1Hr1G000900.5 were identified (Table 10). For two resistance genes i.e. HORVU1Hr1G000840.1 and HORVU1Hr1G000910.9 no SNP/InDel was identified.

Functional annotation of defined SNPs between parental lines, MBR1012 and Scarlett, also revealed synonymous mutations for 11 SNPs whereas for 17 SNPs amino acid substitutions were detected. For two SNPs the arginine amino acid changed to a stop codon (TGA) (Table 11). Multiple alignment also revealed polymorphisms between the parents and the barley reference sequence (Appendix 14).

Mapping of two polymorphic markers resulted in shortening the target interval from 0.07 cM to 0.05 cM genetically and from 0.44 Mb to 0.35 Mb physically. Two new recombinations where identified between gene based marker QBScg134 and the previously mapped SNP QBS98. Finally, the number of candidate genes in target interval was reduced from 29 genes to 23 genes.

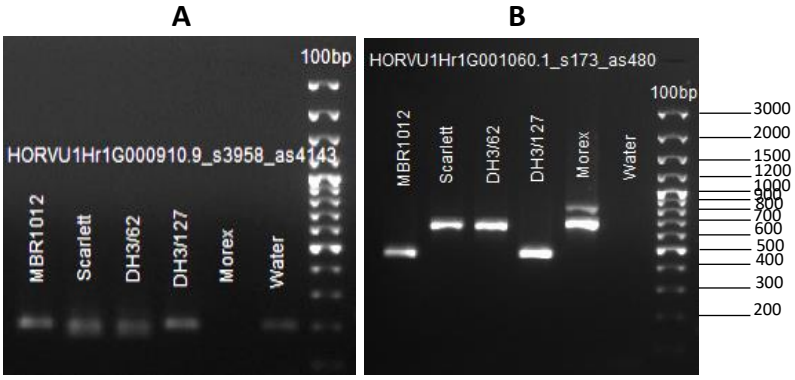

Figure 15. PCR product of two size polymorphic markers originated from two candidate genes on parental lines, two DH lines and Morex. A: gene, HORVU1Hr1G000910.9, MBR1012 fragment, ca. 200bp and Scarlett fragments, ca. 186bp. B: gene, HORVU1Hr1G001060.1, MBR1012 fragment, ca. 500bp and Scarlett fragments, ca. 700 bp. DNA ladder (molecular-weight size marker): 100bp.

Table 10. Low-pass resequencing of 29 genes from the candidate interval.

	Resistance gene	Gene	Position Chr. 1H	Gene size (bp)/Morex CDS	Designed primer pairs	Product size (bp)/Morex genome	Product size (bp)/Morex CDS	PCR products in parents	Sequenced fragments	Size of sequenced region (bp)		SNP	InDel	presence /absence PCR products	Percent of re-sequenced length in MBR1012 and Scarlett
										MBR1012	Scarlett				
LC															
Gene 1	No	HORVU1Hr1G000820.1	2206515-2208709	2194	2	-	761	single band in parents	2	713	713	0	0		32.49
Gene 2	No	HORVU1Hr1G000870.1	2315379-2318239	2860	1	-	186	single band in parents	1	187	187	No SNPs; partly heterozygous	0		6.53
Gene 3	No	HORVU1Hr1G000880.1	2321017-2321809	792	1	-	204	single band in parents	1	206	206	2	0		26.01
Gene 4	No	HORVU1Hr1G000890.2	2322023-2323245	1222	1	-	253	multiple bands	0	-	-	0	0		0
Gene 5	No	HORVU1Hr1G000970.1	2480502-2481189	687	1	-	371	single band in parents	1	199	199	1 SNP; only forward primer sequence	0		28.96
Gene 6	No	HORVU1Hr1G000980.1	2508528-2508781	253	1	-	185	multiple bands	0	-	-	0	0		0
Gene 7	No	HORVU1Hr1G001000.1	2519412-2519796	384	1	-	154	only on Scarlett	0	-	-	0	0	Dominant for Scarlett	0
Gene 8	No	HORVU1Hr1G001010.1	2523769-2523985	216	1	-	0	No band in parents	0	-	-	0	0		0
Gene 9	No	HORVU1Hr1G001070.1	2563416-2564658	1242	1	-	168	single band in parents	1	167	167	2	0		31.44
Gene 10	No	HORVU1Hr1G001100.1	2608865-2611347	2482	2	-	415	single band in parents	2	402	402	2	0		16.19
Gene 11	No	HORVU1Hr1G001110.1	2631559-2632063	504	1	-	171	single band in parents	1	114	114	2 SNPs; only forward primer sequence	0		22.61
HC															
Gene 1	Yes	HORVU1Hr1G000830.3	2237274-2244749	7475	8	5726	-	4 primers with multiple band/ 3 primers with single band in parents/ one primer only MBR1012	4	2257	2257	7	0	One primer dominant for MBR1012	30.16
Gene 2	Yes	HORVU1Hr1G000840.1	2256321-2260574	4253	2	594	-	one primer with single band in parents/ one primer only MBR1012	1	264	264	0	0	One primer dominant for MBR1012	6.207
Gene 3	No	HORVU1Hr1G000850.2	2288429-2288693	264	1	-	164	single band in parents	1	162	162	No SNPs; partly heterozygous	0		61.36

Table 10. continued

	Resistance gene	Gene	Position Chr. 1H	Gene size (bp)/Morex CDS	Designed primer pairs	Product size (bp)/Morex genome	Product size (bp)/Morex CDS	PCR products in parents	Sequenced fragments	Size of sequenced region (bp)		SNP	InDel	presence /absence PCR products	Percent of re-sequenced length in MBR1012 and Scarlett
										MBR1012	Scarlett				
Gene 4	Yes	HORVU1Hr1G000860.7	2302070-2309447	7377	7	5531	-	6 primers with single band in parents/ one primer with smear	7	4629	4629	9	0		62.74
Gene 5	Yes	HORVU1Hr1G000900.5	2323680-2330056	6376	10	7520	-	5 primers with single band in parents/ 3 primers with multiple bands/one primer only MBR1012/one primer only Scarlett	6	3643	3308	Many SNPs	35 Indels with 1 to three bp/ large deletion in Scarlett	One primer dominant for MBR1012/one primer dominant for MBR1012	57.13
Gene 6	Yes	HORVU1Hr1G000910.9	2362586-2367013	4427	8	5313	-	4 primers with single band in parents/ 3 primers with multiple bands	6	1629	1629	0	0		36.79
Gene 7	No	HORVU1Hr1G000920.2	2415651-2417084	1433	1	-	323	single band in parents	1	325	325	3	0		22.67
Gene 8	No	HORVU1Hr1G000930.1	2429752-2430871	1119	1	-	268	single band in parents	1	270	270	7	0		24.12
Gene 9	No	HORVU1Hr1G000940.3	2471775-2475305	3530	1	-	371	multiple bands	0	-	-	0	0		0
Gene 10	No	HORVU1Hr1G000960.10	2477273-2481215	3942	1	-	376	single band in parents	1	726	729	6	3 Indels		18.49
Gene 11	No	HORVU1Hr1G001020.1	2524728-2525648	920	1	-	381	single band in parents	1	360	360	0	0		39.13
Gene 12	No	HORVU1Hr1G001030.1	2532014-2532317	303	1	-	154	week band	0	-	-	0	0		0
Gene 13	No	HORVU1Hr1G001040.1	2534715-2535433	718	1	-	266	single band in parents	1	269	269	3	0		37.46
Gene 14	No	HORVU1Hr1G001050.1	2551018-2551266	248	1	-	108	multiple bands	0	-	-	0	0		0
Gene 15	No	HORVU1Hr1G001060.1	2558952-2560347	1395	1	-	308	single band in parents	1	438	626	4	188bp deletion in MBR1012		44.87
Gene 16	No	HORVU1Hr1G001080.1	2578526-2578814	288	1	-	149	single band in parents	1	147	147	3	0		51.04
Gene 17	No	HORVU1Hr1G001090.1	2585277-2589625	4348	1	-	352	multiple bands	0	-	-	0	0		0
Gene 18	No	HORVU1Hr1G001120.2	2649700-2763382	113682	1	-	342	multiple bands	0	-	-	0	0		0
Total				174934	61	24684	6430			17107	16963				9.88

Table 11. Functional annotation of SNPs between parental lines (MBR1012 and Scarlett) originated from candidate genes located within the 0.44Mb of target interval.

Gene	Alignment position	Type of mutation	Codon	Amino acid substitution	Mutation/SNP			
					Cultivar: MBR1012		Cultivar: Scarlett	
					Position	Nucleotide	Position	Nucleotide
LC								
HORVU1Hr1G000880	197	E	TTG->TTT	L->F	122	A	122	C
	250	E	CGA->TGA	R->*	175	A	175	G
HORVU1Hr1G000970	482	E	AGA->TGA	R->*	187	T	187	A
HORVU1Hr1G001100	1779	I			219	A	219	G
	1780	I			220	T	220	A
HC								
HORVU1Hr1G000830	3405	E	GCA->GCC	synonymous	39	T	39	G
	3573	E	GAT->GAC	synonymous	207	G	207	A
	3782	E	AGT->CGT	S->R	78	G	78	T
	4521	E	TTT->TTC	synonymous	231	A	231	G
	4736	E	GAG->AAG	E->K	446	C	446	T
	4854	U			564	G	564	C
	4887	U			597	A	597	G
HORVU1Hr1G000860	2639	I			153	T	153	C
	2651	I			165	A	165	G
	2799	E	CAG->CAA	synonymous	308	C	308	T
	2834	E	TCT->GCT	S->A	343	C	343	A
	2908	E	AGT->ATT	S->I	417	A	417	C
	2917	E	CGA->CAA	R->Q	426	C	426	T
	2967	E	CTG->CTT	synonymous	476	A	476	C
	3534	E	CTC->CTT	synonymous	79	A	79	G
	3785	E	ACA->GCA	T->A	330	T	330	C
HORVU1Hr1G000920	1091	E	CCC->CCT	synonymous	99	G	99	A
	1112	E	ACT->ACA	synonymous	120	A	120	T
	1185	E	GGC->GCC	G->A	193	G	193	C
HORVU1Hr1G000930	171	E	CCG->TCG	P->S	44	A	44	G
	207	E	CTG->GTG	L->V	80	C	80	G
	218	E	GTT->GCT	V->A	91	G	91	A
	230	E	CTT->CAT	L->H	103	A	103	T
	245	E	CAC->CTC	H->L	118	A	118	T
	266	E	CAG->CGG	Q->R	139	T	139	C
	271	E	GTG->GTA	synonymous	144	T	144	C
	930	E	CGG->CAG	R->Q	260	C	260	T
HORVU1Hr1G000960	1071	I			401	G	401	A
	1082	I			412	G	412	T
	1337	I			156	T	156	A
	1341	I			160	A	160	G
	1379	I			198	A	198	G
HORVU1Hr1G001040	90	E	GAC->GAT	synonymous	22	T	22	A

Table 11. continued

Gene	Alignment position	Type of mutation	Codon	Amino acid substitution	Mutation/SNP			
					Cultivar: MBR1012		Cultivar: Scarlett	
					Position	Nucleotide	Position	Nucleotide
HORVU1Hr1G001040	100	E	AAC->ATC	N->I	32	G	32	A
	213	E	GCC->GCT	synonymous	145	A	145	G
	615	E	GGA->CGA	G->R	112	G	112	C
HORVU1Hr1G001060	635	I			132	T	132	A
	651	I			148	G	148	A
	714	I			211	T	211	C

*: Stop codon

E: Exonic

I: Intronic

U: Upstream

D:Downstream

3.5 Development of diagnostic markers for the *Rph$_{MBR1012}$* gene

Diagnostic assessment of markers co-segregating markers with the *Rph$_{MBR1012}$* was conducted. The number of alleles detected varied from two alleles for markers QBS116, QBS117, QBS128, QBS130, GBS546, QBS98 and GBS626, seven alleles for GBR534, eight alleles for QBScg134, thirteen alleles for GBMS187 and fourteen alleles for QBS2. Two markers, GBS626 (83.60%) and GBS546 (80.32%) correlated to a high degree with the *Rph$_{MBR1012}$* resistance phenotype in the set of varieties analysed. These two markers revealed in 51 and 49 cultivar/lines, the same allele as the susceptible line Scarlett, and displayed the same allele as in the resistant parent MBR1012 only in 10 and 12 cultivars/lines respectively. Moreover, marker GBMS187 with 98.41% accuracy for *Rph$_{MBR1012,}$* due to the dominant mode of inheritance is of limited value. Therefore, GBS626 and GBS546 are considered as the best markers for marker-assisted-selection of *Rph$_{MBR1012}$* owing to their co-dominant manner and robustness. QBS117 with 9.8% accuracies for *Rph$_{MBR1012}$* has no diagnostic value to target this gene (Table 12).

Table 12. Selected Bowman lines and parental lines carrying 25 known *Rph* genes analysed for the diagnostic value of the developed markers linked to the resistance locus *Rph*$_{MBR1012}$. Size of alleles and restriction patterns.

Cultivar/lines	*Rph-Gene*	Gene Locus	QBS128	QBS116	QBS117	GBS626	GBR534	GBS546	GBS564	GBMS187	QBS98	QBS2	QBScg134
MBR1012	Resistant	1HS	T	C	C	300_400	358	490	354_368	Null	1000	361	390
Scarlett	Susceptible	-	C	T	T	400	Null	330	349-367	161	900	328	475
Oderbruker	*Rph1*	2H	C	C	C	400	358	330	349-352-368	181	1000	328	500-575
B.L.195-246-1	*Rph1*	2H	H	C	C	400	358	330	349-352-368	178	1000	328-361	390-475
Peruvian	*Rph2*	5HS	T	C	C	300-400	358	490	354-368	178	1000	374	390
B.L.195-266-1	*Rph2*	5HS	C	T	C	400	358	330	349-367	178	1000	361	-
B.L.193-343-1	*Rph2*	5HS	T	C	C	400	358	330	346-366	148/178	1000	328	390
Estate	*Rph3*	7HL	T	C	C	300-400	358	330-490	354-368	178	1000	328	390
B.L.195-267-2	*Rph3*	7HL	C	T	C	400	358	330	349-367	178	1000	328-361	390
Gold	*Rph4*	1HS	C	C	C	400	358	490	354-368	178	1000	327	390
B.L.195-268-4	*Rph4*	1HS	C	C	C	400	358	490	354-368	178	1000	328	390
Magnif	*Rph 5*	3HS	T	C	C	300-400	358	490	352-365	164	1000	-	390
B.L.195-269-1	*Rph5*	3HS	T	C	C	300_400	352-358	490	351-365	178	1000	361	390
Bolivia	*Rph2+6*	5HS+3 HS	T	C	C	300-400	358	490	354-368	178	1000	321-341	390
B.L.195-270-2	*Rph6*	3HS	C	T	C	400	358	330	349-365	178	1000	361	390
Cebad capa	*Rph 7*	3HS	T	C	C	400	358	330	349-367	178	-	327	390
B.L.193-21	*Rph7*	3HS	C	T	C	400	358	330	349-367	178	1000	361	390
B.L.196-424-1	*Rph7*	3HS	H	C	H	400	338-358	330	365	178	1000	321	390-550
Egypt4	*Rph8*	7HS	C	C	C	400	358	330	349-367	178	1000	328-373	500-575
B.L.195-349-4	*Rph.8h*	7HS	H	C	C	400	358	330	349-367	178	1000	328	475-550
Trumph	*Rph12*	5HL	C	C	C	400	358	490	349-367	181	1000	328-361	380-450-475
B.L.194-224	*Rph9*	5HS	C	T	C	400	358	330	349-367	178	1000	361	390
B.L.195-274-1	*Rph9*	5HS	C	T	C	400	358	330	366	178	1000	361	390
BC8	*Rph 10*	3HL	T	C	C	300-400	358	330-490	354-368	167	1000	351	390
B.L.195-272-1	*Rph10*	3HL	C	T	C	400	358	330	349-367	178	1000	323	390

Table 12. continued

Cultivar/lines	*Rph-Gene*	Gene Locus	QBS128	QBS116	QBS117	GBS626	GBR534	GBS546	GBS564	GBMS187	QBS98	QBS2	QBScg134
BC67	*Rph 11*	6HS	T	C	C	300-400	358	330-490	354-368	161	1000	328-351	390
B.L.195-273-2	*Rph11*	6HS	C	T	C	400	358	330	349-367	178	1000	361	390
195-288-2	*Rph13*	7HS	C	T	C	400	358	330	368	178	1000	328-361	390
195-290-2	*Rph14*	7HS	C	T	C	400	358	330	349-365	148/178	1000	361	390
B.L.195-282-2	*Rph15*	2HS	C	T	C	400	358	330	349-367	178	1000	361	390
Hordeum spontaneum/680	*Rph16*	2HS	C	-	C	400	358	330	365	181	-	328	390
NGB22914	*Rph 17*	2HS	C	C	C	400	358	490	354-368	160	1000	328	390
NGB22900	*Rph 18*	2HL	H	C	C	400	358	330-490	349-367	162	1000	359	380-450-475
Prior	*Rph 19*	7HL	T	C	C	300-400	358	330-490	354-368	165	1000	351	390
Flagship	*Rph 20*	6H	H	C	C	400	358	330-490	349-367	163	1000	328-361	390-450-475
Ricardo	*Rph 21*	4H	T	C	C	400	358	330	354-368	162	1000	320	390
NGB22893	*Rph 22*	2HL	T	C	C	400	358	330	349-367	165	1000	328	390
Yerong	*Rph 23*	7HS	T	C	T	400	Null	330	349-367	166	900	320	475
ND24260-1	*Rph 24*	5HS	T	C	T	400	Null	330	349-367	165	-	368	390
Fongtien	*Rph 25*	5HL	H	C	C	400	358	330	352-368	165	1000	328	475-550
Reka1	*Rph3+?*	7HL+?	T	C	C	300-400	358	490	351-365	181	900	328	390
HOR4280	*Rph1d+1r*	2H	-	T	C	400	358	330	366	181	900	328	475
Bowman	*Susceptible*	-	-	T	C	400	358	330	349-365	176	1000	361	390
Bowman	*Rph15*	2HS	C	T	C	400	349-358	330	349-365	176	1000	361	390
HOR500-1	*Rph1d+1r*	2H	C	-	C	400	358	490	365	178	-	328	390
Grossklappige	*Susceptible*	-	T	C	H	400	338-358	330	352-366	148/178	900	328	390-525
Sudan	*Rph1*	2H	H	C	C	400	351-358	330	351-365	178	1000	328	475-550
Quinn	*Rph2+5*	5HS+3 HS	T	C	T	400	338-358	330	349-365	178	900	321-327-363	475
Rika × F_1	*Rph3*	7HL	T	C	C	400	352-358	330	352	178/161	-	361	550
Lada	*Susceptible*	-	C	T	C	400	358	490	349-365	181	-	-	475
Krona	*Rph12*	5HL	H	C	C	400	358	330	349-365	181	1000	361	390-475

Table 12. continued

Cultivar/lines	*Rph-Gene*	Gene Locus	QBS128	QBS116	QBS117	GBS626	GBR534	GBS546	GBS564	GBMS187	QBS98	QBS2	QBScg134
Alexis	*Susceptible*	-	C	T	C	400	358	330	365	178	1000	328	390
HOR679-3	*Rph3*	7HL	C	C	C	400	352-358	330	352-365	178	-	361	390
Vada	*Partial res.*	-	C	C	C	400	352-358	490	351-365	178	1000	325	390
HOR1132	*Rph2r*	5HS	T	C	C	300-400	352	330-490	352-365	178	1000	328-361	390
HOR1063	*Partial res.*	-	C	T	C	400	358	330-490	352-365	148	1000	361	390
Salome	*Susceptible*	-	C	T	C	400	358	330	349-366	178	-	-	475
HOR2596	*Rph9*	5HS	C	C	C	400	358	330	349-366	178	1000	-	550
Emir	*Susceptible*	-	H	C	C	400	358	330-490	349-366	178	1000	361	390-475
Karat	*Susceptible*	-	T	C	C	400	352-358	330	352	178	-	361	550
L94	*Susceptible*	-	C	T	C	400	349-358	330	349-365	178	-	-	475
MBR532	*Susceptible*	-	H	C	C	400	358	330	351-365	178	1000	325	475-550
Igri	*Susceptible*	-	C	H	T	400	338-358	330	350-366	161/178	900	318	475
Diagnostic value			67.21	36.06	9.8	83.6	24.59	80.32	65.57	100	26.22	72.13	40.98

4 Discussion

Puccinia hordei (*Ph*), causing leaf rust, is an important pathogen in temperate barley-growing areas worldwide (Chen et al. 1994, Mathre 1982). It causes yield losses of up to 62% and a reduced grain quality by limiting the photosynthetic area and by diverting assimilates (Cotterill et al. 1992a, Line 2002, Helfer 2014). *P. hordei* can be controlled by timely fungicide application, but the most economical, environmental, and consumer-friendly approach is breeding of resistant cultivars (Park et al. 2015). However, disease resistance provided by major *Rph* genes is often overcome due to the emergence of new virulent *P. hordei* pathotypes (Niks 1982, Steffenson et al. 1993, Park 2003) indicating the need for introducing new sources of resistance into barley breeding. It is also important to isolate the respective resistance genes towards deciphering the structure and function offering the possibility of developing functional markers for breeding and create new alleles by e.g. CRISPR/Cas9 (Kumar et al. 2018). In the present thesis, the *Puccinia hordei* resistance gene $Rph_{MBR1012}$ was targeted with respect to high resolution mapping and finally isolation via map based cloning. $Rph_{MBR1012}$ was mapped in the telomeric region of chromosome 1HS (König et al. 2012).

Previously, Perovic et al. (2001) have shown that the barley landrace MBR1012 is resistant to the barley leaf rust isolate I-80. The respective gene was latter mapped using 14 SSRs and three SNP-markers on barley chromosome 1HS (König et al. 2012). A null allele of the SSR marker GBMS187 was identified as the closest linked marker at 0.8 cM proximal to the resistance gene. The allelic status of $Rph_{MBR1012}$ and *Rph4* (McDaniel and Hathcock 1969), two genes mapped on the short arm on barley chromosome 1HS, still has to be elucidated (Perovic et al, in preparation). Meanwhile, a set of 19 allele-specific PCR-based genetic markers was developed and along with other markers used to saturate $Rph_{MBR1012}$ (Fazlikhani et al. 2019). In addition different staining methods were applied to deeper understand the plant-pathogen interaction encoded by the $Rph_{MBR1012}$ resistance gene.

4.1 Advanced genomic resources greatly facilitate gene isolation in barley

In this study, marker saturation was conducted for high-density and high-resolution genetic mapping of the resistance gene $Rph_{MBR1012}$ on the basis of the previously defined mapping interval (Perovic et al. 2012). For many years, mapping of resistance genes relied on the use of various molecular markers i.e. RFLPs, RAPDs, AFLPs and SSRs (Williams et al. 1990, Graner et al. 1991, Chalmers et al. 1993, Kleinhofs et al. 1993, Vos et al. 1995, Qi et al. 1998, Ramsay et al. 2000, Varshney et al. 2007b). For instance, the powdery mildew resistance gene *mlo* which was the first gene, isolated by map-based cloning in barley, was isolated by a combined use of RFLP and AFLP markers (Büschges et al. 1997). Today, the physical-genetic and functional sequence assembly of

the barley genome spanning 4.98 Gb (IBSC 2012), population sequencing (POPSEQ) with more than 2 million SNPs (Mascher et al. 2013a, Ariyadasa et al. 2014), the 9K Illumina iSelect Custom Genotyping Array (Comadran et al. 2012), the 50K Illumina Infinium array (Bayer et al. 2017), exome capture data (Mascher et al. 2013b, Mascher et al. 2014), as well as the reference sequence of barley (Mascher et al. 2017), provide extraordinary valuable sources for the development of molecular markers and for positional cloning. SNP markers have become the markers of choice for plant molecular genetics and gene discovery due to the genome-wide abundance, today (Hansson et al. 2018). Even though SNPs are less informative due to their bi-allelic nature in comparison to SSR markers, this is compensated by their high abundance and easy detection in high-throughput screening methods (Kumar et al. 2012). In present study, 56 polymorphic markers from different sources were developed for high-resolution mapping of $Rph_{MBR1012}$. In addition to the 37 markers originated from the Genome zipper (Mayer et al. 2011) and 9K iSelect array (Comadran et al. 2012), four markers were developed from the 50K iSelect Illumina array (Bayer et al. 2017). This newly developed array consist of 44,040 SNPs markers, of which 6,251 are from the 9K iSelect chip. The majority of the 50K markers has a precise physical position and gene annotation in the barley pseudomolecule assembly (Bayer et al. 2017). Bykova et al. (2017) also used the 50K iSelect illumina array to identify SNPs associated with resistance to two isolates of spot blotch (Ch3 and Kr2) in barley. They reported 27 and 3 SNPs associated with resistance to Kr2 and Ch3 , respectively (Bykova et al. 2017). Likewise, the current study highlights the use of the GBS technology for the construction of a high-density linkage map of a barley RIL population. For further saturation of the target region, the SNPs identified by GBS could be easily converted to PCR based KASP markers. The low cost and high quality of generated sequence data that are compatible with other high-throughput genotyping platforms render GBS a cost-effective alternative to other whole-genome genotyping platforms (Elshire et al. 2011). In addition, for crops with large genomes like barley, this technique is technically less challenging compared to exome sequencing owing to reduced sample handling and few PCR and purification steps, making it a fast approach (He et al. 2014). This technology was also used to construct the high-density linkage map of flag leaf traits in bread wheat (Hussain et al. 2017). In barley, e.g. GBS was applied to discover informative SNPs in the vicinity of the *Rha2* resistance gene against cereal cyst nematode (Van Gansbeke et al. 2019).

Finally using the above mentioned advanced marker resources and technologies resulted in down-sizing the resistance locus $Rph_{MBR1012}$ to 0.07 CM. Eleven co-segregating markers were identified at the target locus. Although the Rph_{MBR10} is located in telomeric region the deviation of the observed recombination frequency from the average predicted value for the telomeric region of the barley chromosomes reveals suppressed recombination regions within this interval with a high number of co-segregating markers. The observed ration of 6.38 Mb/cM in the target interval

of *Rph*$_{MBR1012}$ indicates a low number of recombination events. In accordance with these data, Kunzel et al. (2000) estimated a ratio of >4.4 Mb/cM for a suppressed recombination.
Kunzel et al. (2000) categorized chromosomes in different sub-regions comprising regions of suppressed recombination (>4.4 Mb/cM), high recombination (1.0–4.4 Mb/cM), and very high recombination (<1.0 Mb/cM). They also showed that the higher recombination events occurred in the distal part of the short and long arms of chromosome 1H. The high number of co-segregating markers and candidate genes clearly show that a high resolution mapping population of 4775 F_2 plants was not sufficient to dissolved the co-segregation region (Fazlikhani et al. 2019). Similarly, Wei et al. (1999) identified a strongly suppressed recombination within a delimited 240-kb interval carrying the barley *Mla* powdery mildew resistance gene cluster on barley chromosome 1H.

4.2 Barley physical map is a fundamental platform for gene identification

In this study, we have shown an efficient use of the barley reference sequence in physical mapping and in marker saturation. Positional gene isolation involves the establishment of a physical map of the part of the chromosome spanning the target gene region that is identified by genetic mapping. Therefore, the position of the target gene in the genome as well as the genetic/physical relation are milestones in the positional gene isolation. It has been shown that small genetic distance around the centromere correspond to a large physical distance (Ma et al. 2010). Prior to the release of barley reference genome, the mapping procedure was typically being continued until two markers flanking the gene of interest hit a single bacterial artificial chromosome (BAC) clone, so-called “chromosome landing” (Tanksley et al. 1995). In case this was not achieved, chromosome walking was principally required to identify overlapping BAC clones and construct the physical contig spanning the target interval. This is in general hampered in cereals by the large amount of repetitive DNA (Flavell et al. 1993). Using sequence information of BAC ends, the BAC library was screened to identify the next adjacent overlapping BAC clone (Stein and Graner 2005). Depending on the size of the gap required to being covered, this procedure was a laborious and time-consuming task. However, by the construction of the barley reference genome and accessibility to the sequence data, there is no need for to invest further efforts into chromosome landing and chromosome walking. It is only required to find recombination events at the target interval to identify a single candidate gene (Gupta and Varshney 2013). By new advances in sequencing, the first draft of the physical map of barley spanning 4.56 Gb, with more than 3.90 Gb anchored to a high-resolution genetic map was published in 2012 (IBSC 2012). Five years later Mascher et al. (2017) released the reference barley genome sequence, which is now the most important genomic resource for gene identification. This reference genome eased the

development of high-throughput genotyping platforms, such as the 9K and 50K iSelect Illumina array (Comadran et al. 2012, Bayer et al. 2017) and is a useful tool for estimating the natural variation in barley (Perovic et al. 2018).

The present work aiming at the identification of the functional gene underlying *Rph*$_{MBR1012}$ conferring resistance to barley leaf rust represents an example for the usefulness of this resource in cloning barley genes. Firstly, comparison of two closely linked markers QBS94 (BOPA1_8670) and QBS113 (BOPA1_7372) to the physical and sequence assembly of the barley genome allowed anchoring the genetic to the physical map of the *Rph*$_{MBR1012}$ locus. This opened the possibilities for an efficient use of the reference sequence for targeted marker development and narrowing down the genetic interval. Anchoring the high-density genetic map to the barley reference sequence resulted in a precise mapping of the markers at the physical level and facilitated obtaining information on the collinearity between the genetic and the physical map as well as to figure out the putative genes located in the target interval. This information positioned the gene in the same region as the previous study (Perovic et al. 2012) but with a lower number of putative candidate gene after shortening the target interval. Due to the large size of target interval, first 29 candidate genes were identified. The discrepancy observed in the distal part of the interval however is due to the non-fixed orientation of the BAC-based sequence contig within the small scaffold having only one anchor point (M. Mascher, pers. comm.). However, this discrepancy as well as the observed cluster of several markers co-segregating with the resistance locus may indicate that the recombination frequency in this interval is suppressed by chromosomal rearrangements. Therefore, a further increase of the genetic resolution to disclose some of the candidates is unlikely and alternative parents may be used.

Surveys of genome reference sequences from various species, including rice, Arabidopsis, and barley, provide information on the widespread occurrence of local rearrangement among *R* genes triggered by the interaction between plants and their pathogens (Meyers et al. 2003, Monosi et al. 2004, IBSC 2012). This is clearly demonstrated by Hanemann et al. (2009) in fine mapping of the *Rrs2* gene conferring resistance to scald in barley. At the genetic resolution provided by 4,721 F_2 plants, the *Rrs2* gene was fine mapped to an interval of 0.08 cM containing several co-segregating markers with the locus.

Extracted information from the barley reference sequence on the other hand facilitated reducing the size of the interval via the development of additional markers directly from identified candidate genes. Therefore, the example of the *Rph*$_{MBR1012}$ illustrates the future potential of gene isolation in barley, and as a consequence the much easier map-based cloning procedure. In similar studies putative candidate genes were identified for *Rha2* (resistance against cereal cyst nematode) (Van Gansbeke et al. 2019), *Ryd3* (tolerance to BYDV), and *MlLa-H* (resistance to the powdery mildew) (Hoseinzadeh et al. 2019) using the barley reference genome.

4.3 Map-based cloning approach as a method of choice in gene isolation

To study the underlying genetic basis of different traits, gene-mapping methods try to find the link between the genotypes of individuals and the phenotypes of interest. Although this approach can be a laborious task, depending on the recombination frequency around the target locus, it became a standard technique to identify underlying genes (Lukowitz et al. 2000). Map-based cloning is an efficient approach in large and complex genomes to clone the genes of interest without prior knowledge and with respect to barley, it became a standard forward genetics approach for gene identification (Stein and Graner 2005). *Mlo,* conferring resistance to the powdery mildew was the first gene isolated by map-based cloning in barley (Büschges et al. 1997). Brueggeman et al. (2002) used map-based cloning to isolate the *Rpg1* gene conferring resistance to barley stem rust (*P. graminis*) which encodes a serine/threonine protein kinase with two tandem kinase domains. Similarly, Bulgarelli et al. (2004) cloned the race-specific resistance gene *Rdg2a* on chromosome 7HS, and validated it by stable transformation of a susceptible variety. *Rdg2a* encodes a NLR protein that resides in a gene cluster with two additional NLR genes with high sequence identity in close proximity (<50 kb). Four years later, *Rpg5* was isolated via map based cloning and it turned out that it encodes a NLR protein with an integrated carboxy-terminal protein kinase domain (Brueggeman et al. 2008). To date, two genes effective against BaMMV/BaYMV have been isolated using a map-based cloning approach. These recessive resistance genes correspond to two different host factors needed for virus accumulation. The *rym1/11* locus encodes a Protein Disulfide Isomerase Like 5-1 (Hv-PDIL5-1) which is speculated to function as a chaperone in correct folding of virus proteins (Verchot 2012, Yang et al. 2014). The *rym4/5* gene encodes an Eukaryotic Translation Initiation Factor 4E (Hv-eIF4E), which putatively functions in assisting the translation initiation of a bymovirus precursor protein (Kanyuka et al. 2005, Stein et al. 2005, Moury et al. 2014, Sanfaçon 2015). Using the same approach in the present study led to the construction of a high-resolution mapping population for $Rph_{MBR1012}$. Consequently, $Rph_{MBR1012}$ was mapped in the same region previously mapped by König et al. (2012) but in a shortened interval.

Besides resistance genes, some of the genes identified by map-based cloning contribute to important and complex agronomic traits such as grain protein content, vernalization requirement, photoperiod sensitivity, tolerance to abiotic stress and morphological and domestication traits (Stein and Graner 2005). These genes often encode transcription factors and they are probably involved in the regulation of the expression of downstream genes.

4.4 Identification of candidate genes

High-resolution mapping procedures alone can only significantly narrow down the target interval and decrease the number of candidate genes, but additional efforts are needed to isolate the gene. Therefore, to construct the physical map of the linked region, YACs (yeast artificial chromosomes) were used in previous time on a large-scale. More recently, stable and easy to handle BACs (bacterial artificial chromosomes) were screened with the markers which are linked e.g. to disease resistance gene (Dixon 2010). Construction of a genomic contiguous sequence scaffold (contig), which is defined as a set of overlapping segments of DNA, is the next step to cover all the genomic elements in the targeted region (Weikuan and Goldowitz 2012). Afterwards, a precise contig has to be sequenced and analysed by different techniques. This procedure commonly is utilized for the identification of potential genes, but is not needed today due to the availability of the reference sequence. Potential candidate genes then should be confirmed using a variety of genetic and biochemical methods. Following above procedure, two closely linked markers QBS127/QBS98 along with all co-segregated markers in target interval of 0.44 Mb were subjected to screening a non-gridded BAC library as most likely the $Rph_{MBR1012}$ locus is not present in the reference sequence. Physical mapping of resistance genes using BAC library has been done for cloning of some resistance genes like, *Rpg4* and *Rpg1* (resistance to barley stem rust) and the *Rrs2* scald resistance gene in barley (Druka et al. 2000, Brueggeman et al. 2002, Mammadov et al. 2006, Hanemann et al. 2009).

Allele specific re-sequencing also is an effective method commonly used to down size the target interval. The re-sequencing analysis of five *R* genes out of 29 genes within the $Rph_{MBR1012}$ interval displays functional polymorphisms, i.e. SNPs and medium and / or large-scale insertions and deletions. Although the comparative sequencing analysis of the putative candidate genes in this target interval would provide the clear evidence on potential candidate gene, further investigations are still required to determine the function of the potential candidate genes, as well as of the other five NBS-LRR genes at the $Rph_{MBR1012}$ locus.

Bulked segregant analysis by sequencing, BSA-seq is another direct approach for gene identification (Klein et al. 2018). It is a very promising alternative to the direct identification of the target mutation (Vlk and Repkova 2017). Whole genome DNA sequencing provides the possibility of sequencing individual plants to perform genome-wide SNP discovery and consequently enables fast-forward genetic mutation identification for reasonable costs (Nishijima et al. 2018). In this regard, most genomic studies use DNA and/or RNA sequencing to accomplish different kinds of functional studies. In 2009, the first report of a successful gene cloning by utilizing a combination of BSA and NGS in plants was published for the model plant Arabidopsis (Schneeberger et al. 2009). Meanwhile, this method was also successfully

implemented for gene isolation in crop plants, e.g. for the characterization of seven agronomic traits (Abe et al. 2012). In 2009, Next-generation sequencing in company with (CRISPR)-Cas9 technology was used for the first time for finding and assembly of plant virus and viroid genome and to develop the resistant plants (Hadidi et al. 2016). Exome capture based re-sequencing has been successfully applied for direct gene identification. As an example, the many-noded dwarf (mnd) gene of barley was recently cloned through an exome capture based mapping-by-sequencing strategy (Mascher et al. 2014). Functional validation of identified candidate genes is the next step towards gene cloning. The gene function analysis can be performed either through the over-expression of the gene of interest or silencing using RNA interference (RNAi) (Abe and Ichikawa 2016) so-called Transient induced gene silencing assay (TIGS). Both approaches have been developed over the years and proven valuable tools for identification the gene function (Ihlow et al. 2008, Douchkov et al. 2014). The TIGS and overexpression constructs can be generated in plasmid vectors pIPKTA9 and pIPKTA30 (Schweizer et al. 1999, Douchkov et al. 2005). It is expected that overexpression of the genes that do not provide resistance on susceptible plants should result in super-susceptibility whereas the overexpression of the responsible gene for the trait on susceptible parent / genotypes leads to resistance. In transient gene silencing, the constructs will be checked in both susceptible and resistant parents to assess their phenotypes (Schweizer et al. 1999). In comparison with the stable transformation, both assays can be performed in 10 days and the function of genes can be assessed without the generation of transgenic plants.

With the new methods for targeted genome modification that are based upon customizable endonucleases, it is possible to functionally study and modify DNA sequences at a previously defined site of choice in the host genome (Koeppel et al. 2019). In plant research, four platforms of customizable endonucleases have been used so far; meganucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the RNA-guided, clustered, regularly interspaced, short palindromic repeats (CRISPR)-associated (Cas) endonucleases (Kim et al. 1996, Christian et al. 2010, Vu et al. 2014). In 2013, Upadhyay et al. (2013) were the first to show that Cas endonucleases are in principle applicable in a Triticeae species. In barley and wheat, Cas endonucleases have been mainly used for the investigation of gene functions by knockout, for the modification of metabolite contents and for increasing resistance to fungal pathogens (Koeppel et al. 2019). The first published use of Cas endonucleases in barley aimed to induce mutations in *HvPM19* (Lawrenson et al. 2015), which encodes an ABA-induced plasma membrane protein previously described in wheat as a positive regulator of dormancy. Holme et al. (2017) used Cas endonuclease-induced mutations to investigate the function of the *PHYTASE GENE A* of barley. *HvPAPhy_a* acts as the main regulator of the phytase content in the barley grain. CRISPR/Cas9 technology also has been used for Microrchidia (MORC) proteins which play a role

in plant immunity against fungal pathogens (Kumar et al. 2018). All together since the CRISPR/Cas9 allows creating mutations in homoeologous genes simultaneously, this method promises a huge potential for the fast creation of new disease resistant cultivars.

Using the transcription activator-like effector nucleases (TALEN) is another approach in functional analysis and genome editing (Kumar et al. 2018). This tool can also delete genetic sequences or silence a gene at a specific location (Rinaldo and Ayliffe 2015). In barley TALEN mediated genome editing technology has been successfully applied for *HvPAPhy_a* (Wendt et al. 2013) and GFP (transgene) (Gurushidze et al. 2014, Budhagatapalli et al. 2015) genes. Another example of using this method has already been reported on all three barley *Mlo* homoeologous of wheat resulting in resistance against powdery mildew (Wang et al. 2014).

Resistance gene enrichment sequencing (RenSeq) is an innovative approach that could be used instead of classical gene mapping (Jupe et al. 2013). It can be used for mapping of resistance loci in segregating populations but also for rapid cloning of *R* genes via its combination with mutagenesis (Mutagenesis and Resistance gene enrichment sequencing: MutRenSeq). The latter approach is especially useful in regions where separation of the resistance locus through recombination is not realistic like in the present study (Steuernagel et al. 2016). Steuernagel et al. (2016) used this method to isolate two wheat stem rust resistance genes, *Sr22* and *Sr45*, which mediate resistance to *Puccinia graminis* f. sp *tritici*. They also designed a NLR bait library for cereals containing 60,000 120-mer RNA probes with ≥95% identity to predicted NLR genes present in Triticeae species like barley (*Hordeum vulgare*), hexaploid bread wheat (*Triticum aestivum*), tetraploid wheat (*T. durum*), wild einkorn (*T. urartu*), domesticated einkorn (*T. monococcum*), and three goatgrass species including *Aegilops tauschii*, *Ae. sharonensis*, and *Ae. speltoides* (Steuernagel et al. 2016).

AgRenSeq, is a combining association genetics with *R* gene enrichment sequencing (Arora et al. 2019). AgRenSeq allows breeders to discover NLR genes in their breeding germplasm and develop gene-specific markers for marker-assisted selection. Thus, this method has a high potential for genetic improvement of most crops and will also provide new fundamental insights into the structure and evolution of species-wide functional *R* gene architectures (Arora et al. 2019). This method was used for cloning *Sr46 and SrTA1662* genes in *Aegilops tauschii* (Arora et al. 2019).

4.5 Plant-pathogen interaction

Although susceptibility is relatively easy to recognize and to score, the expression of the resistance phenotype is not always that much obvious due to the abortion of fungal development during the formation of haustorial mother cells by plant defence like HR in biotrophic fungi. In

the present investigations on the barley leaf rust interaction we aimed at elucidating the reaction associated with the HR governed by *Rph*$_{MBR1012}$. Several mechanisms have been suggested to account for the reduced ramification of fungal development in resistant plants. Our investigation showed that the resistance response was associated with a cell wall reinforcement through accumulation of phenolic compounds and reactive oxygen species (ROS) production like hydrogen peroxide (H_2O_2), which are typically seen in race-specific resistance responses, i.e. the so called HR (Jones and Dangl 2006). The increase of phenolic compounds accumulation contributes to the fluorescence associated with the resistant interactions (Mayama and Shishiyama 1978, Toyoda et al. 1978, Aist and Israel 1986). Similar histological observations about the hypersensitive cell death have been noted for *Blumeria graminis* f. sp. *hordei* (Hückelhoven et al. 1999). It has been shown that *Rph18* from (38P18) derived from a cross between *H. vulgare* and *H. bulbosum* also seems to confer HR (Johnston et al. 2013). Barley genotype Cebada Capa, also possesses one major gene (*Rph7*) for race-specific HR and several QTL for non-hypersensitive partial resistance to *P. hordei* (Parlevliet and van Ommeren 1985, Qi et al. 2000).

While HR is a common reaction of resistance gene-mediated defense, there are a few known cases of NBS-LRR genes conferring resistance without HR. For instance, the resistance conferred by *Rph20* is not associated with a HR in seedlings and adult plants. *Rph20* conferring APR to *P. hordei* is characterized as a non-hypersensitive or minor gene APR (Golegaonkar et al. 2009b, Hickey et al. 2011, Singh et al. 2013). Johnston et al. (2013) indicated, that *the H. bulbosum* introgression in line '182Q20' carrying *Rph22*, confers a very high level of resistance to *P. hordei* at the seedling stage, which is not HR-based. Bulgarelli et al. (2010) have shown the absence of HR associated with resistance to the barley *Mla1* powdery mildew resistance gene and *Rx* gene in potato conferring potato virus x (PVX). This may be due to the rapid resistance mechanism inhibiting the accumulation of the avirulence factor to levels that would otherwise trigger a more extensive host response (Bendahmane et al. 1999).

Our experiments have not only shown a positive stain of H_2O_2, but also indicated the presence of phenolic substances. Auto-fluorescence and phenolic compounds are commonly associated with incompatible host-pathogen interactions (Kulbat 2016). Accumulation of phenolic compounds was observed at 24 hpi after inoculation in the resistant genotype MBR1012. During the time course of infection, cell death spread to adjacent non-invaded cells to abort further growth of infection hyphae of pathogen. This spread of cell death is also found in plants resistant to other rust fungi and to other obligate biotrophs (Heath 1981, Silva et al. 2002). Exhibition of autofluorescence of mesophyll cells in resistant plants is indicative for the presence of phenolic-like compounds. Most studies approve that the accumulation of phenolics and lignification may be associated with cell death, thus being one of the first reactions of plant defense against

infection (Cohen et al. 1990). Similarly, the H_2O_2-dependent DAB staining results from the dying epidermal cells as early as 7 dpi after inoculation in the resistant parent. The presence of DAB staining underneath appressoria known as an early response to attack occurring at around the time of attempted penetration of the cell wall by the fungal penetration peg (Vanacker et al. 2000). Consequently, in common with many other investigations our results support the view that the failure of barley leaf rust to establish a biotrophic relationship in resistant plants presumably is due to the HR controlled by $Rph_{MBR1012}$.

5 Outlook

So far, a high-resolution mapping population of 537 segmental RILs was constructed and used to saturate the *Rph*$_{MBR1012}$ region with a new set of molecular markers. In order to increase the map resolution even more, the enlargement of the population by screening a new set of F_2 plants is required. The results obtained in this study, which led to the development of diagnostic molecular markers for *Rph*$_{MBR1012}$, are very useful for barley breeders, who now can employ these markers in their breeding programs. Nevertheless, the identification and cloning of *Rph*$_{MBR1012}$ still is of relevant scientific interest.

The implementation of three different staining methods provided information regarding host-pathogen interactions of barley and *Puccinia hordei* and provide hints on function of the resistance gene active in MBR1012.

Established methods will now allow studying the candidate genes detected in the target interval in more detail. In this context, the following steps are proposed for the identification of for the resistance gene *Rph*$_{MBR1012}$:

I. Screening a new set of F_2 plants for enhancing the resolution followed by mapping the new SNPs and InDel derived from respective candidate genes to downsize the target interval. Use of a new cross between MBR1012 as resistant parent and a susceptible parent as an alternative cross, to overcome the lake of recombination in target interval of original cross (MBR1012×Scarlett).
II. The sequence gap spanning the *Rph*$_{MBR1012}$ region needs to be saturated by additional molecular markers. Allele specific resequencing of the candidate genes is a cost effective method for markers saturation as well as the development of marker assays based on the already identified SNP and InDels from genes located in the candidate region.
III. Screening of BAC the library present for MBR1012 in order to construct a physical map of the donor plant.
IV. Construction of the physical map of *Rph*$_{MBR1012}$ on donor line MBR1012. This purpose could be possible by anchoring the generated BAC contigs and the flanking markers on the genetic map.
V. Finally, a confirmation of candidate genes, by using site directed mutagenesis, e.g. CRISPR/Cas9.

6 Summary

The barley leaf rust (*Puccinia hordei* G. Otth) disease seriously threatens barley production worldwide. Improving natural resistance to *Puccinia hordei* in cultivars is a practical way to prevent major yield losses. Symptoms of barley leaf rust may range from small chlorotic flecks to large pustules containing spores. The pathogen can sexually recombine and mutate frequently resulting in new virulent strains. Since resistant varieties have been proven to be only durable for a certain amount of time, identification of new and effective sources of resistance genes in barley followed by their use in breeding is of great interest. Until now, twenty-six race-specific genes (*Rph1* to *Rph26*) have been already identified (Yu et al. 2018).

Therefore the aims of this thesis were: (i) to develop a high-resolution mapping population for the resistance gene $Rph_{MBR1012}$ resistance, (ii) to saturate the locus using all available state-of-the-art genomic resources, (iii) to anchor the genetic map to the barley reference sequence (iv) to characterize the putative candidate rust resistance genes by allele specific re-sequencing, (v) to test the developed markers for their diagnostic value and (vi) to conducted histopathological analysis on the race-specific resistance encoded by $Rph_{MBR1012}$.

A high resolution mapping population of 537 RILs was constructed by analysing 4775 F_2 plants by flanking markers BOPA1_8670/QBS94 and BOPA1_7372/QBS113 corresponding to a resolution of 0.01% recombination. Inoculation of seedlings of 537 segmental RILs in the greenhouse with isolate I-80, resulted to a segregation of 261 resistant: 276 susceptible (χ^2 1:1 = 0.4189, df = 1, $p < 0.05$) indicating a monogenic inheritance of resistance.

Different molecular marker sources utilizing the Genome Zipper, Illumina 9K iSelect and the Infinium 50K chips as well Genotyping-by-sequencing (GBS) technology were implemented and 56 SSRs and SNP markers were developed for marker saturation. Additionally, allele specific re-sequencing of candidate genes located in target interval resulted in two size polymorphism markers. Finally, the target interval was shortened to 0.05 cM. Eleven markers were detected co-segregating with $Rph_{MBR1012}$. All mapped markers were anchored to the barley reference genome available on (http://webblast.ipk-gatersleben.de/barley_ibsc/). Eight low-confidence and 15 high-confidence genes were defined in target region comprising 0.35 Mb including five disease resistance genes.

To elucidate the diagnostic value of eleven closely linked markers, a subset of 25 barley genotypes/lines carrying *Rph1* to *Rph25* resistance genes, some parental lines as well as 15 introgression Bowman lines carrying *Rph1* to *Rph15* were used. The results showed that two SNP markers GBS546 and GBS626 are considered as the best diagnostic markers for marker-assisted-selection of $Rph_{MBR1012}$ due to their co-dominance and robustness.

Allele specific re-sequencing of high and low confidence genes located in target interval resulted in 17,107 bp in MBR1012 and 16,963 bp, 259 SNPs were identified for disease resistance genes from the target interval.

Utilization of different staining showed that resistance to the barley leaf rust in resistant lines is most likely due to the HR controlled by $Rph_{MBR1012}$. More over these methods might improve the accuracy of phenotyping procedures

In summary, results of the present study demonstrated the usefulness of genomic resources and the availability of the barley reference sequence for gene isolation in barley.

7 Zusammenfassung

Zwergrost (*Puccinia hordei* G. Otth) verursacht weltweit hohe Ertragseinbußen in der Gerstenproduktion. Die Verbesserung der Resistenz gegenüber *Puccinia hordei* in Kultivaren ist ein praktischer Weg, um diese Ertragsverluste zu vermeiden. Die Symptome von Zwergrost reichen von kleinen chlorotischen Flecken bis zu großen sporulierenden Pusteln. Der Erreger kann sich sexuell vermehren und währenddessen mutieren, was zu neuen virulenten Stämmen führt. Da die in Sorten eingebrachten Resistenzen gegen Zwergrost durch diese neuen virulenten Stämme überwunden werden können, ist die Identifizierung neuer und wirksamer Quellen für Resistenzgene in Gerste und deren Verwendung in der Züchtung von großem Interesse. Bisher wurden 26 rassenspezifische Gene (*Rph1* bis *Rph26*) identifiziert (Kavanagh et al. 2017). Ziel dieser Arbeit war es daher: (i) eine hochauflösende Kartierungspopulation für das Resistenzgen $Rph_{MBR1012}$ zu entwickeln, (ii) den Locus der Resistenz mit allen verfügbaren genomischen Ressourcen abzusättigen, (iii) die genetischen Karte in der Gerstenreferenzsequenz zu verankern, (iv) die mutmaßlichen Kandidatengene für die Rostresistenz durch allelspezifische Re-Sequenzierung zu charakterisieren, (v) die entwickelten Marker auf ihren diagnostischen Wert zu testen und (vi) histopathologische Analysen der rassenspezifischen Resistenz $Rph_{MBR1012}$ durchzuführen.

Durch die Analyse von 4775 F_2-Pflanzen der Kreuzung MBR1012 x Scarlett mit den flankierenden Markern BOPA1_8670 / QBS94 und BOPA1_7372 / QBS113 wurde eine hochauflösende Kartierungspopulation von 537 RILs konstruiert. Dies entspricht einer Auflösung von 0,01% Rekombination. Die Inokulation dieser 537 segmentalen RILs im Gewächshaus mit dem Zwergrostisolat I-80 führte zu einer Segregation von 261 resistenten zu 276 anfälligen RILs (χ^2 1:1 = 0.4189, df = 1, $p < 0.05$). Dies weist auf eine monogene Vererbung der Resistenz hin.

Mit Hilfe verschiedener genomischer Ressourcen, wie dem Genome Zipper, dem Illumina 9K iSelect- und dem Infinium 50K-Chip, sowie der Genotyping-by-Sequencing Technologie (GBS), wurden 56 SSR- und SNP-Marker für die Markerabsättigung entwickelt. Zusätzlich führte die allelspezifische Neu-Sequenzierung von Kandidatengenen, welche sich im Zielintervall befinden, zu zwei Größen-Polymorphismus-Markern. Schließlich wurde das Zielintervall auf 0,05 cM verkleinert, dabei wurden elf Marker nachgewiesen, die mit $Rph_{MBR1012}$ co-segregieren. Alle kartierten Marker wurden im Gerstenreferenzgenom verankert, welches unter (http://webblast.ipk-gatersleben.de/barley_ibsc/) verfügbar ist. Acht HC- und 15 LC-Gene, inklusive fünf Krankheitsresistenzgenen, wurden in der Zielregion von 0,35 Mb definiert. Um den diagnostischen Wert der elf co-segregierenden Marker aufzuklären, wurde ein Set bestehend aus 25 Gerstengenotypen/ -linien, welche die Resistenzgene *Rph1* bis *Rph25* tragen, einigen Elternlinien, sowie 15 Bowman-Introgressions-Linien, welche die Resistenzgene *Rph1* bis *Rph15*

tragen, zusammengestellt. Die Ergebnisse zeigten, dass die zwei SNP-Marker GBS546 und GBS626 aufgrund ihrer Co-Dominanz und Robustheit die besten diagnostischen Marker für die markergestützte Selektion von $Rph_{MBR1012}$ sind. Die allelspezifische Re-Sequenzierung von Genen mit hohem und niedrigem Konfidenzniveau im Zielintervall ergab eine Größe von 17.107 Basenpaaren im resistenten Kreuzungselter MBR1012 und 16.963 Basenpaaren im anfälligen Kreuzungselter Scarlett. Dabei wurden 259 SNPs in den Sequenzen der fünf Krankheitsresistenzgene innerhalb des Zielintervalls identifiziert.

Die Verwendung verschiedener Färbungen für die histopathologische Analyse zeigte, dass die Resistenz gegen Zwergrost in den resistenten Genotypen höchstwahrscheinlich auf die von $Rph_{MBR1012}$ kontrollierte hypersensitive Reaktion zurückzuführen ist. Darüber hinaus können diese Methoden die Genauigkeit von Phänotypisierungsverfahren verbessern.

Zusammenfassend zeigten die Ergebnisse dieser Studie den Nutzen genomischer Ressourcen, sowie die Verfügbarkeit der Gerstenreferenzsequenz, für die Genisolierung in Gerste.

8 References

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9 Appendix

Appendix 1. Composition of general PCR reaction.

Reagent (producer)	Concentration	Preparation volume
HPLC gradient grade water		6.22 µl
BD buffer	10 x	1.0 µl
$MgCl_2$	25 mM	1.0 µl
dNTP Set	10 mM	0.2 µl
Primer forward	10 pmol μl^{-1}	0.25 µl
Primer reverse	10 pmol μl^{-1}	0.25 µl
Polymerase FIRE Pol	5 U μl^{-1}	0.08 µl
DNA	25 ng μl^{-1}	1.0 µl
		10 µl

Appendix 2. Composition of PCR reaction for ABI analysis.

Reagent (producer)	Concentration	Preparation volume
HPLC gradient grade water		6.12 µl
BD buffer	10 x	1.0 µl
$MgCl_2$	25 mM	1.0 µl
dNTP Set	10 mM	0.2 µl
M13 tail	10 mM	0.1 µl
Primer forward	1 pmol μl^{-1}	0.25 µl
Primer reverse	10 pmol μl^{-1}	0.25 µl
Polymerase FIRE Pol	5 U μl^{-1}	0.08 µl
DNA	25 ng μl^{-1}	1.0 µl
		10 µl

Appendix 3. Initial PCR-Program.

PCR Program	Temperature	Time	Cycles	Primer Pairs
Touchdown 62°C-56°C	94°C	5min	1 cycle	All primer pairs
	94°C	30 s		
	62°C	30 s	12 cycles (-0.5°C)	
	72°C	30 s		
	94°C	30 s		
	56°C	30 s	35 cycles	
	72°C	30 s		
	72°C	10 min	1 cycle	
	4°C	20 min	1 cycle	
	15°C	∞	1 cycle	

Appendix 4. Optimized PCR-Programs.

PCR Program	Temperature	Time	Cycles	Primer Pairs
Touchdown	94°C	5min	1 cycle	contig1008249_s216 / as1256
62°C-56°C	94°C	30 s		BOPA2_31144_ctig_54745_s80_as349
	62°C	30 s	12 cycles (0.5°C)	contig1008249_s6 / as1304
	72°C	1 min		GZ54_contig_2547982_s282_as487
	94°C	30 s		GZ29_contig_161159_s1073_as1968
	56°C	30 s	35 cycles	contig247169_s465 / as1513
	72°C	1 min		contig2160218_s20 / as250
	72°C	10 min	1 cycle	contig1019464_s215 / as1264
	4°C	20 min	1 cycle	contig1019464_s115 / as1400
	15°C	∞	1 cycle	contig1031142_s338_as1388
				contig_1019464_s164_as1479
				contig_1019464_s17_as1310
				contig121251_s111_as1150
				GZ59_contig_45711_s3862_as4545
				GZ59_contig_s45711_s976_as1572
				contig247169_s465_as1513
				GZ66_contig370643_s7768_as8867
				GZ67_contig_66602_s8296_as_9813
				GZ67_contig_66602_s8931_a_s9813
				QBS2
				MBR564
				MBR546
				GBR534
				MBR626
2 PCR-Program				
Touchdown	94°C	5 min	1 cycle	BOPA7174-contig127650_s31_as336
62°C-56°C	94°C	30 s		
	62°C	30 s	12 cycles (0.5°C)	
	72°C	1 min		
	94°C	30 s		
	56°C	30 s	55 cycles	
	72°C	1 min		
	72°C	10 min	1 cycle	
	4°C	20 min	1 cycle	
	15°C	∞	1 cycle	
3 PCR-Program				
Touchdown	94°C	5 min	1 cycle	
62°C-56°C	94°C	30 s		BOPA1_8670_388_s22_as141
	62°C	30 s	12 cycles (0.5°C)	contig_64079_s49_as297
	72°C	30 s		QBS2_GBR218_s49_as361
	94°C	30 s		149683_ctig_224474_s8_as303

	56°C	30 s	55 cycles	232577_ctig_1007221_s20_as318
	72°C	30 s		BOPA1_7372
	72°C	10 min	1 cycle	GBMS187
	4°C	20 min	1 cycle	
	15°C	∞	1 cycle	

4 PCR-Program

58°C	94°C	5 min	1 cycle	contig1008249_s315 / as1362
	94°C	30 s		contig1008249_s133 / as1186
	58°C	30 s	38 cycles	contig53937_s351 / as1405
	72°C	1 min		contig145384_s22 / as1066
	72°C	10 min	1 cycle	
	4°C	20 min	1 cycle	
	15°C	∞	1 cycle	

5 PCR-Program

Touchdown	94°C	5 min	1 cycle	GZ29_contig_161159_s90_as1968
62°C-56°C	94°C	30 s		
	62°C	30 s	12 cycles (0.5°C)	
	72°C	2 min		
	94°C	30 s		
	56°C	30 s	35 cycles	
	72°C	2 min		
	72°C	10 min	1 cycle	
	4°C	20 min	1 cycle	
	15°C	∞	1 cycle	

Appendix 5. Buffers and solutions for gel electrophoresis.

Reagent	**Concentration in solution**
Loading dye (10 x)	
Glycerol	40 %
Bromophenol blue	3 %
EDTA	0.2 M
TBE buffer (10 x)	
Tris / HCl	89 mM
Boric acid (pH 8.3)	89 mM
EDTA	2.5 mM
Ethidium bromide	
3,8-Diamino-5-ethyl-6-phenylphenanthridinium bromide	10 mg ml^{-1}

Appendix 6. Enzyme digestion protocol.

Reagents	Preparation volume
H_2O (HPLC)	7.9 µl
buffer	2 µl
Enzyme	0.1 µl
PCR Product	(8-)10 µl
	20 µl

Appendix 7. Developed CAPS markers with corresponding restriction enzymes, cleavage sites, incubation time/temperature as well as composition of restriction digest.

Marker Name	Restriction enzyme cleavage site	Incubation time and temperature	Incubation time and temperature
contig247169	***Hpy*CH4IV** (BioLabs)	3 h 37°C	PCR product
s465/ as1513	5'...A↓CGT...3'		HPLC gradient grade water
	3'...TGC↑A...5'		10x buffer NEB1 (BioLabs)
			Restriction enzyme
contig53937	***Bam*HI** (Fermentas)	3 h 37°C	PCR product
s351 / as1405	5'...G↓GATCC...3'		HPLC gradient grade water
	3'...CCTAG↑G...5'		buffer tango (Fermentas)
			Restriction enzyme
contig1008249	***Bse*NI** (*Bsr*I) (Fermentas)	3 h 65°C	PCR product
s133 / as1186	5'...ACTGGN↓...3'		HPLC gradient grade water
	3'...TGAC↑CN...5'		10x buffer B (Fermentas)
			Restriction enzyme
contig1008249	***Hpa*II** (Fermentas)	3 h 37°C	PCR product
s315 / as1362	5'...C↓CGG...3'		HPLC gradient grade water
contig_1019464	3'...GGC↑C...5'		10x buffer tango (Fermentas)
s17 / as1310			Restriction enzyme
contig1019464	***Mfe*I** (*Mun*I) (Fermentas)	3 h 37°C	PCR product
s215 / as1264	5'...C↓AATTG...3'		HPLC gradient grade water
	3'...GTTAA↑C...5'		10x buffer G (Fermentas)
			Restriction enzyme
contig121251	***Taa*I**	3 h 65°C	PCR product
s111 / 1150	(HpyCH4III)(Fermentas)		HPLC gradient grade water
	5'...A CN↓G T...3'		10x buffer tango (Fermentas)
	3'...T G↑NC A...5'		Restriction enzyme
contig145384	***Fsp*BI** (*Bfa*I) (Fermentas)	3 h 37°C	PCR product
s22 / as1066	5'...C↓TAG..3'		HPLC gradient grade water
GZ67_contig_66602	3'...GAT↑C...5'		10x buffer tango (Fermentas)
s8931 / as9813			Restriction enzyme

Appendix 7. continued

Marker Name	Restriction enzyme cleavage site	Incubation time and temperature	Incubation time and temperature
contig2160218	***Bsu*RI** (*Hae*III**)**	3 h 37°C	PCR product
s20 / as250	(Fermentas)		HPLC gradient grade water
	5'…GG↓CC…3'		10x buffer R (Fermentas)
	3'…CC↑GG…5'		Restriction enzyme
contig64079	***Sty*I** (*Eco*130I)	3 h 37°C	PCR product
s49 / as297	(Fermentas)		HPLC gradient grade water
	5'…C↓CWWGG…3'		10x buffer 0 (Fermentas)
	3'…GGWWC↑C…5'		Restriction enzyme
MBR546	***Hha*I**	3 h 37°C	PCR product
GZ54_contig_2547982	5'…GCG↓C…3'		HPLC gradient grade water
s282 / as487	3'…C↑GCG…5'		10x buffer tango (Fermentas)
contig1019464			Restriction enzyme
s115 / as1400			
contig2160218	***Bsu*RI** (*Hae*III**)**	3 h 37°C	PCR product
s20 / as250	(Fermentas)		HPLC gradient grade water
	5'…GG↓CC…3'		10x buffer R (Fermentas)
	3'…CC↑GG…5'		Restriction enzyme
contig64079	***Sty*I** (*Eco*130I)	3 h 37°C	PCR product
s49 / as297	(Fermentas)		HPLC gradient grade water
	5'…C↓CWWGG…3'		10x buffer 0 (Fermentas)
	3'…GGWWC↑C…5'		Restriction enzyme
MBR546	***Hha*I**	3 h 37°C	PCR product
GZ54_contig_2547982	5'…GCG↓C…3'		HPLC gradient grade water
s282 / as487	3'…C↑GCG…5'		10x buffer tango (Fermentas)
contig1019464			Restriction enzyme
s115 / as1400			
BOPA2_31144_ctig_54745s	***AciI*** *(SsiI)*	3 h 37°C	PCR product
80 / as349	5'...C↓CGC...3'		HPLC gradient grade water
	3'...GGC↑G...5'		10x buffer 0 (Fermentas)
			Restriction enzyme
contig_1008249	***MlyI***	3 h 37°C	PCR product
s6 / as1304	5'...GAGTCN5↓...3'		HPLC gradient grade water
	3'...CTCAGN5↑...5'		10x buffer tango (Fermentas)
			Restriction enzyme
contig1019464	***TaqI***	3 h 65°C	PCR product
s164 / as1479	5'...T↓CGA...3'		HPLC gradient grade water
	3'...AGC↑T...5'		10x buffer TaqI (Fermentas)
			Restriction enzyme

Appendix 7. continued

Marker Name	Restriction enzyme cleavage site	Incubation time and temperature	Incubation time and temperature
3101_111_s6_as135	***Eco31I*** *(BsaI)* 5'...GGTCTCN1↓...3' 3'...CCAGAGN5↑...5'	3 h 37°C	PCR product HPLC gradient grade water 10x buffer G (Fermentas) Restriction enzyme
GZ59_contig45711 s976 / as1572	***Eco47I*** *(AvaII)* 5'...G↓GWCC...3' 3'...CCWG↑G...5'	3 h 37°C	PCR product HPLC gradient grade water 10x buffer R (Fermentas) Restriction enzyme
149683_ctig224474 s8 / as303	***HpyF10VI*** 5'...GCNNNNN↓NNGC...3' 3'...CGNN↑NNNNNCG...5'	3 h 37°C	PCR product HPLC gradient grade water 10x buffer tango (Fermentas) Restriction enzyme
232577_ctig1007221 s20 / as318	***AjiI*** *(BmgBI)* 5'...CAC↓GTC...3' 3'...GTG↑CAG...5'	3 h 37°C	PCR product HPLC gradient grade water 10x buffer AjiI (Fermentas) Restriction enzyme
GZ67_contig66602 s8296 /as9813	***SspI*** 5...AAT↓ATT...3 ' 3...TTA↑TAA ...5 '	3 h 37°C	PCR product HPLC gradient grade water 10x buffer G (Fermentas) Restriction enzyme
GBS626	***Btscl*** 5'GGATGNN↓3' 3'CCTAC↑NN5'	3 h 55°C	PCR product HPLC gradient grade water 10x buffer G (Fermentas) Restriction enzyme

Appendix 8. Primer combinations, marker type and corresponding restriction enzymes for 9K iSelect and genome zipper markers.

Marker	Marker type	Endonuclease for CAPS markers	Reference
QBS105_3101_111_s6_as135	CAPS	*Eco31I* (*BsaI*)	(Perovic et al. 2012)
QBS95_30969_ctig_121098_s22_as316	CAPS	*Hind III*	(Perovic et al. 2012)
QBS104_BOPA2_ctig_54745_s80_as349	CAPS	*AciI* (*SsiI*)	(Perovic et al. 2012)
QBS108_149683_ctig_224474_s8_as303	CAPS	*HpyF10VI*	(Perovic et al. 2012)
QBS109_232577_ctig_1007221_s20_as318	CAPS	*AjiI* (*BmgBI*)	(Perovic et al. 2012)
QBS70_contig_64079_s49_as297	CAPS	*Eco 130I*	(Rauser 2012)
QBS72_contig_1008249_s315_as1362	CAPS	*HpaII* (*MspI*)	(Rauser 2012)
QBS73_contig_1008249_s133_as1186	CAPS	*BseN1* (*BsrI*)	(Rauser 2012)
QBS74_contig_2160218_s20_as250	CAPS	*HaeIII*	(Rauser 2012)
QBS97_contig_1008249_s6_as1304	CAPS	*MlyI*	(Perovic et al. 2012)

Appendix 8. continued

Marker	Marker type	Endonuclease for CAPS markers	Reference
QBS75_contig_1019464_s215_as1264	CAPS	*MfeI*	(Rauser 2012)
QBS101_contig_1019464_s115_as1400	CAPS	*HhaI*	(Perovic et al. 2012)
QBS102_contig_1019464_s164_as1479	CAPS	*TaqI*	(Perovic et al. 2012)
QBS103_contig_1019464_s17_as1310	CAPS	*HpaII*	(Perovic et al. 2012)
QBS76_contig53937_s351_as1405	CAPS	*BamHI*	(Rauser 2012)
QBS77_contig145384_s22_as1066	CAPS	*BfaI*	(Rauser 2012)
QBS79_contig121251_s111_as1150	CAPS	*TaaI*	(Rauser 2012)
QBS80_contig247169_s465_as1513	CAPS	*Hpy CH4IV*	(Rauser 2012)
QBS111_GZ67_BO_contig_66602_s8296_as_9813	CAPS	*SspI*	(Perovic et al. 2012)
QBS112_GZ67_BO_contig_66602_s8931_as_s9813	CAPS	*BfaI*	(Perovic et al. 2012)
QBS100_GZ54_M_contig_2547982_s282_as487	CAPS	*HhaI* /*DdeI*	(Perovic et al. 2012)
QBS107_GZ59_M_contig_s45711_s976_as1572	CAPS	*Eco47I* (*AvaII*)	(Perovic et al. 2012)
GBS626	CAPS	*BtsCI*	(Perovic et al. 2012)
GBS546	CAPS	*HhaI*	(Kota et al. 2008)
QBS71_contig1031142_s338_as1388	Size polymorphism		(Rauser 2012)
QBS98_GZ29_M_contig_161159_s1073_as1968	Size polymorphism		(Perovic et al. 2012)
QBS99_GZ29_M_contig_161159_s90_as1968	Size polymorphism		(Perovic et al. 2012)
QBS106_GZ59_M_contig_45711_s3862_as4545	Size polymorphism		(Perovic et al. 2012)
QBS94_8670_388_s22_as141	Size polymorphism		(Perovic et al. 2012)
QBS113_BOPA1_7372_139	Size polymorphism		(Perovic et al. 2012)
QBS2_GBR218_s49_as361	Size polymorphism		(Stein et al. 2007, König et al. 2012)
GBS564	Size polymorphism		(Perovic et al. 2012)
GBR534	Size polymorphism		(Perovic et al. 2012)
GBMS187	+/-		(Li et al. 2003)
QBS78_contig50849_s216_as1256	+/-		(Rauser 2012)
QBS110_GZ66_B_contig370643_s7768_as8867	+/-		(Perovic et al. 2012)
QBS96_BOPA7174-365_contig127650_s31_as336	+/-		(Perovic et al. 2012)

Appendix 9. Designed primer pairs for low pass sequencing of 29 candidate genes located in target interval of 0.44 Mb.

	Gene	Position in 1H	Primer	product size	sequence	bases	tm
LC genes	**HORVU1Hr1G000820.1**	**2206515-2208709**	HORVU1Hr1G000820.1_s373	385	CTTTACGCTGATGATGCTGCC	21	60
			HORVU1Hr1G000820.1_as757		TGATAGCGGTCAGGAGGAAGA	21	60.06
			HORVU1Hr1G000820.1_s1031	376	CGACTCGTGTTACTATCGGGG	21	60
			HORVU1Hr1G000820.1_as1406		GCCCACGTTTTCCAGACAATC	21	60.07
	HORVU1Hr1G000870.1	**2315379-2318239**	HORVU1Hr1G000870.1_s13	186	CACCTAGTGCACATCCCCAT	20	59.45
			HORVU1Hr1G000870.1_as199		AGTGCTCTGTCGTGGATGCC	20	62.51
	HORVU1Hr1G000880.1	**2321017-2321809**	HORVU1Hr1G000880.1_s139	204	TGCGTTGAGAAGCTCCATTCA	21	60.27
			HORVU1Hr1G000880.1_as342		CTAACAGATGGTGCAGGGGAG	21	60.13
	HORVU1Hr1G000890.2	**2322023-2323245**	HORVU1Hr1G000890.2_s8	253	TCGATACTATGCGGTCCAACA	21	58.97
			HORVU1Hr1G000890.2_as260		TTTGAACTGAATGCCACGTCA	21	58.71
	HORVU1Hr1G000970.1	**2480502-2481189**	HORVU1Hr1G000970.1_s6	371	CAATTACCATCGTCGTCCTTG	21	57.07
			HORVU1Hr1G000970.1_as377		TCCCGCTCAATCTCAATCTGG	21	59.86
	HORVU1Hr1G000980.1	**2508528-2508781**	HORVU1Hr1G000980.1_s2	185	TTTCCACAACAACCCTTCCCA	21	59.99
			HORVU1Hr1G000980.1_as186		TGCTGCAAAATAGATGGATGAACA	24	59.29
	HORVU1Hr1G001000.1	**2519412-2519796**	HORVU1Hr1G001000.1_s4	154	GCACCACCTCTTTCCTTCTTT	21	58.41
			HORVU1Hr1G001000.1_as158		ACCAATCCATCATGAAGACCTT	22	57.4
	HORVU1Hr1G001010.1	**2523769-2523985**	HORVU1Hr1G001010.1_s29	90	TGTTGTGGGTATGGTTGTTGCT	22	60.69
			HORVU1Hr1G001010.1_as119		ACCAATCCACCATGAAGACCT	21	59
	HORVU1Hr1G001070.1	**2563416-2564658**	HORVU1Hr1G001070.1_s22	168	CACCCTGTTTACTCCCGCATA	21	59.79
			HORVU1Hr1G001070.1_as189		AGCAGAGTCATCAAAACAACAGC	23	60
	HORVU1Hr1G001100.1	**2608865-2611347**	HORVU1Hr1G001100.1_s171	256	AAAGCTGTGGCATCCAGACA	20	59.89
			HORVU1Hr1G001100.1_as426		AGCAGACGAACCATCCACAG	20	60.04
			HORVU1Hr1G001100.1_s468	159	TGATGACGTGGATGGCAATGA	21	60.07
			HORVU1Hr1G001100.1_as627		ACCCCTAGTTTTGCCGAGAGT	21	61.11
	HORVU1Hr1G001110.1	**2631559-2632063**	HORVU1Hr1G001110.1_s81	171	GTTGTACCCTTCCCTGCTAAAG	22	58.66
			HORVU1Hr1G001110.1_as252		TGCACACAACAACAACAACA	20	57.2

Appendix 9. continued

	Gene	Position in 1H	Primer	product size	sequence	bases	tm
HC genes	**HORVU1Hr1G000830.3**	**2237274-2244749**	HORVU1Hr1G000830.3_s114	268	GCACACGATTCTGAAACGCA	20	59.77
			HORVU1Hr1G000830.3_as380		CCCATTCTCTGACGGAACACA	21	60
			HORVU1Hr1G000830.3_s3635	276	TGCGGTTACTTCGTTGTGGA	20	59.9
			HORVU1Hr1G000830.3_as3910		GGAGAGTCCCACCCAACATG	20	60.04
	HORVU1Hr1G000840.1	**2256321-2260574**	HORVU1Hr1G000840.1_s51	154	GATGAGCAAGGCGTCCAACTC	21	61.6
			HORVU1Hr1G000840.1_as204		TTTCGCCCCATCTCTATGCTC	21	59.93
	HORVU1Hr1G000850.2	**2288429-2288693**	HORVU1Hr1G000850.2_s58	164	ATCAGGTGGCTGGAGGAGTC	20	60.98
			HORVU1Hr1G000850.2_as221		GAGACCTGGATGAGCTTGAGG	21	59.86
	HORVU1Hr1G000860.7	**2302070-2309447**	HORVU1Hr1G000860.7_s4406	212	GCACTCATCCATCCCATCCC	20	60.25
			HORVU1Hr1G000860.7_as4617		TCACGGACTAGCCTGGGTAT	20	59.74
			HORVU1Hr1G000860.7_s690	201	GTTGCTGCTTTGGGTCTGTG	20	59.97
			HORVU1Hr1G000860.7_as890		AGCTTTTCCCACTGGCGTAA	20	59.89
	HORVU1Hr1G000900.5	**2323680-2330056**	HORVU1Hr1G000900.5_s42	272	GTCCATGCTGATGAACAAGGC	21	59.87
			HORVU1Hr1G000900.5_as313		TGTTGTAGTGCCCATCCTTCC	21	60
			HORVU1Hr1G000900.5_s4575	319	CGCAAGGGGAACTAAAGCTGT	21	60.88
			HORVU1Hr1G000900.5_as4893		TGTTTCTTCCCTGTTTTCCTGC	22	59.31
			HORVU1Hr1G000900.5_s5012	162	GGTGTTGGTGAGATGTTCAGTT	22	58.79
			HORVU1Hr1G000900.5_as5173		TCATCGAGCAGCCTACCATAG	21	59.11
	HORVU1Hr1G000910.9	**2362586-2367013**	HORVU1Hr1G000910.9_s3958	186	GGCTGCCCTGCTATAAAGAAG	21	58.77
			HORVU1Hr1G000910.9_as4143		GCCATTACTGCAAACATCACA	21	57.43
			HORVU1Hr1G000910.9_s255	228	AATTGCCAGCAGATCCAGACA	21	59.99
			HORVU1Hr1G000910.9_as482		CTCTTAGCCAGGCAGTTCAC	20	58.27
	HORVU1Hr1G000920.2	**2415651-2417084**	HORVU1Hr1G000920.2_s15	323	CCGTGTCCTGTCCGTCATTTT	21	60.88
			HORVU1Hr1G000920.2_as337		CCTCGAACACCATGCTGAAGT	21	60.61
	HORVU1Hr1G000930.1	**2429752-2430871**	HORVU1Hr1G000930.1_s241	268	TTGCAAGAACAACCCCAACGA	21	60.96
			HORVU1Hr1G000930.1_as508		CAACTCTCGTGTCAATGCCGA	21	60.93
	HORVU1Hr1G000940.3	**2471775-2475305**	HORVU1Hr1G000940.3_s142	371	CGAGCAAACAACCGTCCTTCT	21	61.14
			HORVU1Hr1G000940.3_as513		CATCGTCGCCTCCTTCTTCTG	21	60.8

Appendix 9. continued

Gene	Position in 1H	Primer	product size	sequence	bases	tm
HORVU1Hr1G000960.10	**2477273-2481215**	HORVU1Hr1G000960.10_s920	376	CGGCCAGTTTACAGAGACGAC	21	61
		HORVU1Hr1G000960.10_as1295		GACGAGCTCTTTGTTCTCCGG	21	61
HORVU1Hr1G001020.1	**2524728-2525648**	HORVU1Hr1G001020.1_s45	381	ACCCTTCCCATCACAACAACC	21	60.48
		HORVU1Hr1G001020.1_as425		AGGGATTGTTCTTGCAGGACG	21	60.61
HORVU1Hr1G001030.1	**2532014-2532317**	HORVU1Hr1G001030.1_s24	154	ACTCCTCGTCATTGTGGCAAC	21	60.88
		HORVU1Hr1G001030.1_as177		TGGTTGTTGTGGGATGGGTTG	21	61.03
HORVU1Hr1G001040.1	**2534715-2535433**	HORVU1Hr1G001040.1_s48	266	ACCACCCAATTCATCAATGGCT	22	60.56
		HORVU1Hr1G001040.1_as314		TAGCACGGAAGGGTACAACC	20	59.39
HORVU1Hr1G001050.1	**2551018-2551266**	HORVU1Hr1G001050.1_s9	108	ATCTCTCAAGGTGGCTGCCG	20	62.25
		HORVU1Hr1G001050.1_as116		GCAACACAAGCGCCTAGACAA	21	61.74
HORVU1Hr1G001060.1	**2558952-2560347**	HORVU1Hr1G001060.1_s173	308	CCTCCAGCAAAGCATCCGATT	21	61.02
		HORVU1Hr1G001060.1_as480		GTTCGGGAGTGCGTTGTACTT	21	60.87
HORVU1Hr1G001080.1	**2578526-2578814**	HORVU1Hr1G001080.1_s127	149	CAACGTATTGCAAGGTCCCAA	21	59.12
		HORVU1Hr1G001080.1_as276		GGGTTGTTCTTGCAGGACGAT	21	60.88
HORVU1Hr1G001090.1	**2585277-2589625**	HORVU1Hr1G001090.1_s668	352	ATGACAGGGTGGTGAAGGACA	21	61.05
		HORVU1Hr1G001090.1_as1019		AGATTGAGCTTGTAGTGGCGC	21	61.01
HORVU1Hr1G001120.2	**2649700-2763382**	HORVU1Hr1G001120.2_s15	342	CATCTTTGCACTCCTCGCCAT	21	61.02
		HORVU1Hr1G001120.2_as356		GGGAGTGGTGTTTGTTGTGGT	21	60.96

Appendix 10. List of selected primer pairs from 29 candidate genes located in target interval for low pass re-sequencing.

	Gen	Primer
LC genes	**HORVU1Hr1G000820.1**	HORVU1Hr1G000820.1_s373
		HORVU1Hr1G000820.1_as757
		HORVU1Hr1G000820.1_s1031
		HORVU1Hr1G000820.1_as1406
	HORVU1Hr1G000870.1	HORVU1Hr1G000870.1_s13
		HORVU1Hr1G000870.1_as199
	HORVU1Hr1G000880.1	HORVU1Hr1G000880.1_s139
		HORVU1Hr1G000880.1_as342
	HORVU1Hr1G000970.1	HORVU1Hr1G000970.1_s6
		HORVU1Hr1G000970.1_as377
	HORVU1Hr1G001070.1	HORVU1Hr1G001070.1_s22
		HORVU1Hr1G001070.1_as189
	HORVU1Hr1G001100.1	HORVU1Hr1G001100.1_s171
		HORVU1Hr1G001100.1_as426
		HORVU1Hr1G001100.1_s468
		HORVU1Hr1G001100.1_as627
	HORVU1Hr1G001110.1	HORVU1Hr1G001110.1_s81
		HORVU1Hr1G001110.1_as252
HC genes	**HORVU1Hr1G000830.3**	HORVU1Hr1G000830.3_s114
		HORVU1Hr1G000830.3_as380
	HORVU1Hr1G000850.2	HORVU1Hr1G000850.2_s58
		HORVU1Hr1G000850.2_as221
	HORVU1Hr1G000860.7	HORVU1Hr1G000860.7_s4406
		HORVU1Hr1G000860.7_as4617
		HORVU1Hr1G000860.7_s690
		HORVU1Hr1G000860.7_as890
	HORVU1Hr1G000900.5	HORVU1Hr1G000900.5_s42
		HORVU1Hr1G000900.5_as313
		HORVU1Hr1G000900.5_s4575
		HORVU1Hr1G000900.5_as4893
		HORVU1Hr1G000900.5_s5012
		HORVU1Hr1G000900.5_as5173
	HORVU1Hr1G000910.9	HORVU1Hr1G000910.9_s3958
		HORVU1Hr1G000910.9_as4143
	HORVU1Hr1G000920.2	HORVU1Hr1G000920.2_s15
		HORVU1Hr1G000920.2_as337
	HORVU1Hr1G000930.1	HORVU1Hr1G000930.1_s241
		HORVU1Hr1G000930.1_as508
	HORVU1Hr1G000960.10	HORVU1Hr1G000960.10_s920
		HORVU1Hr1G000960.10_as1295
	HORVU1Hr1G001020.1	HORVU1Hr1G001020.1_s45
		HORVU1Hr1G001020.1_as425
	HORVU1Hr1G001040.1	HORVU1Hr1G001040.1_s48
		HORVU1Hr1G001040.1_as314
	HORVU1Hr1G001060.1	HORVU1Hr1G001060.1_s173
		HORVU1Hr1G001060.1_as480
	HORVU1Hr1G001080.1	HORVU1Hr1G001080.1_s127
		HORVU1Hr1G001080.1_as276

Appendix 11. Designed primer pars of five disease resistance genes located in target interval.

Gene/Function	physical position/bp	Primer	product size	sequence	bases	tm
HORVU1Hr1G000830.3 Disease resistance protein	**2237274-2244749**	morex_contig_53491_s11553	801	ACTTCATACGCTGTTCCAACG	21	58.93
		morex_contig_127934_as139		TTCGTGTCTGGATCTGCTGG	20	59.75
		morex_contig_127934_s38	850	GTGTCCAGTCAGTTCAGGCA	20	59.89
		morex_contig_127934_as887		TGGCACAGAAAGCAAAGCAC	20	59.9
		morex_contig_127934_s740	866	CTTCGTACCAAGACCAGCGT	20	60.04
		morex_contig_127934_as1605		CCTGGAGGCATGCTCTTCAA	20	60.03
		morex_contig_127934_s1549	659	TTCCCTCTGCCACCTCTACA	20	59.88
		morex_contig_127934_as2207		TTAGGGCAGGAAACGCTGAG	20	60.04
		morex_contig_127934_s2132	981	CCTGAACCACAATTGCTGCA	20	59.32
		morex_contig_127934_as3112		GAACTCTGACATGCCCTGCT	20	60.04
		morex_contig_127934_s2965	1025	CCTGCCAGGTCTTGAGTCTC	20	59.75
		morex_contig_127934_as3989		TCCATGGCGTTTCTCTGTGG	20	60.32
HORVU1Hr1G000840.1 Powdery mildew resistance protein PM3 variant	**2256321-2260574**	morex_contig_51818_ap_s3431	440	ACGGCTTGCAGAATCAGACA	20	59.96
		morex_contig_51818_ap_as3870		AGTTCACTTGACTTGGGCGT	20	59.82
HORVU1Hr1G000860.7 Disease resistance protein	**2302070-2309447**	morex_contig_55033 _ap_s985	951	ACCTCTCGAGATTTTCCATGCA	22	59.76
		morex_contig_51818 _ap_as115		AGGTTCTTCTTGCGGTGAGG	20	59.96
		morex_contig_51818 _ap_s51	1076	TCATGGGTGAGGATGGCAAC	20	60.03
		morex_contig_51818 _ap_as1126		GCTGACAACGTTACCTGAAGC	21	59.81
		morex_contig_51818 _ap_s980	995	GTCTCTTCGAGTGCATCCCA	20	59.47
		morex_contig_51818 _ap_as1974		AACCTGCAAGTGTTGGACCT	20	59.74
		morex_contig_51818 _ap_s1882	1155	AGTGTAGAGGTGACGGAGCA	20	60.25
		morex_contig_51818 _ap_as3036		CTTCAAAAGTTGGTCAGCGGG	21	60
		morex_contig_51818 _ap_s2932	941	ACCATGCAAGCAGACCTTCA	20	59.89
		morex_contig_51818 _ap_as3872		GCAGTTCACTTGACTTGGGC	20	59.69
HORVU1Hr1G000900.5 Disease resistance protein	**2323680-2330056**	morex_contig_51837 _ap_s2714	719	TCTTCAGCAGCCACCATGTT	20	59.89
		morex_contig_51837 _ap_as3432		GGGCGCAGAGTAGTCAGTAC	20	59.9

Appendix 11. continued

Gene/Function	physical position/bp	Primer	product size	sequence	bases	tm
		morex_contig_51837 _ap_s3290	848	CACTTACGCCAGACAGCAGA	20	60.04
		morex_contig_51837 _ap_as4137		ACCACGTAGAAAGGGCATCG	20	60.11
		morex_contig_51837 _ap_s3999	1186	GCCAGCGTCTTCTCATCAGT	20	60.11
		morex_contig_51837 _ap_as5184		TGTGCACGTTTGGTAGAGATGT	22	60.22
		morex_contig_51837 _ap_s5063	1125	CCTTCAACGGACTCTGGCAT	20	60.04
		morex_contig_51837 _ap_as6187		TGGAGGAGAGTGCATGGGTA	20	59.96
		morex_contig_51837 _ap_s5924	993	TGCAACAATGGTGCTTCAGG	20	59.32
		morex_contig_51837 _ap_as6916		TGTGACCATGAAGCCAAGTGA	21	59.86
		morex_contig_51837 _ap_s6788	956	AGTGTTTGAATGGCAGGGGA	20	59.52
		morex_contig_51837 _ap_as7743		GATGTGAACTCCCTGGCCAA	20	59.96
		morex_contig_51837 _ap_s7558	940	ACCATGCAAGCAGACCTTCA	20	59.89
		morex_contig_51837 _ap_as8497		TGTGCTGCGGGAGTTACTTT	20	59.89
HORVU1Hr1G000910.9 Disease resistance protein	**2362586-2367013**	morex_contig_54254_ap_s5647	975	GCAAACAGAATCCATGGCGT	20	59.47
		morex_contig_54254_ap_as6621		TGTTCAATGTCCCGGCATCT	20	59.67
		morex_contig_54254_ap_s6525	1085	ATGAACTCGGACATGCCCTG	20	60.11
		morex_contig_54254_ap_as7609		TGGGATGCAGTGGAAGAGAC	20	59.38
		morex_contig_54254_ap_s7489	1018	AAGGTCCTTGCAGCAATGGT	20	60.18
		morex_contig_54254_ap_as8506		CATGCCTTGAAGCTCTGCCT	20	60.68
		morex_contig_54254_ap_s8358	991	AGGTCCAACACTTGCAGGTT	20	59.74
		morex_contig_54254_ap_as9348		CCGGTAGTGCAGTGTTGACA	20	60.25
		morex_contig_54254_ap_s9151	830	AGCCACGACATCTCTTCACA	20	59.03
		morex_contig_158429 _ap_as5741		CCGTGAAGCCAGGAAGAAGG	20	60.67
		or/morex_contig_54254_ap_s9151	857	AGCCACGACATCTCTTCACA	20	59.03
		morex_contig_158429 _ap_as5768		CGACGAATTCAAGTACGAAGCA	22	59.34

Appendix 12. List of selected primer pairs for re-sequencing of disease resistance genes in target interval.

Gene/Function	Primer
HORVU1Hr1G000830.3 Disease resistance protein	morex_contig_53491_s11553
	morex_contig_127934_as139
	morex_contig_127934_s740
	morex_contig_127934_as1605
HORVU1Hr1G000840.1 Powdery mildew resistance protein	morex_contig_51818_ap_s3431
	morex_contig_51818_ap_as3870
HORVU1Hr1G000860.7 Disease resistance protein	morex_contig_55033 _ap_s985
	morex_contig_51818 _ap_as115
	morex_contig_51818 _ap_s51
	morex_contig_51818 _ap_as1126
	morex_contig_51818 _ap_s980
	morex_contig_51818 _ap_as1974
	morex_contig_51818 _ap_s2932
	morex_contig_51818 _ap_as3872
	morex_contig_51837 _ap_s3290
	morex_contig_51837 _ap_as4137
	morex_contig_51837 _ap_s3999
	morex_contig_51837 _ap_as5184
HORVU1Hr1G000910.9 Disease resistance protein	morex_contig_54254_ap_s5647
	morex_contig_54254_ap_as6621
	morex_contig_54254_ap_s9151
	morex_contig_158429 _ap_as5741
	oder/morex_contig_54254_ap_s9151
	morex_contig_158429 _ap_as5768

Appendix 13. Designed primer pairs for whole length sequencing of five disease resistance genes located in target interval of 0.44 Mb.

Gene	Position in 1H	Primer	product size	sequence	bases	tm
HORVU1Hr1G000830.3	**2237274-2244749**	HORVU830.3_s2648	731	TGTTCATACGCTGTTCCAACG	20	59.2
		HORVU830.3_as3379		AATCGGTTTGCCTCCACTCC	21	60.32
		HORVU830.3_s3322	850	TGGGTTGAGGCAGACATTCT	20	58.93
		HORVU830.3_as4172		GCTTCATGTGTGGTGGCAAA	20	59.61
		HORVU830.3_s4064	841	GTGGAAGAATGGGAGGCCAT	20	59.74
		HORVU830.3_as4905		ATGCTCTTCAACTCCGGACA	20	59.02
		HORVU830.3_s4657	826	GCATGCCTTGAAGCTCTGTTT	21	59.73
		HORVU830.3_as5483		TATAACCGCCTTCGCCACAA	20	59.75
		HORVU830.3_s5361	983	AGAGGCATGAAGAACGGACA	20	59.02
		HORVU830.3_as6344		GAGATGCCGGGACCTTGAAC	20	60.74
		HORVU830.3_s6109	731	TCTTCAAGAGTTGGCAATTGGT	22	58.43
		HORVU830.3_as6840		CCACTGTTGCCAATAATGTCCAG	23	60.12
		HORVU830.3_s6716		GCAAGAGAGCATTTACTTTCCCC	23	59.87
HORVU1Hr1G000840.1	**2256321-2260574**	HORVU840.1_s4	308	TTCGTCGAAGACTTCATTTGCC	22	59.52
		HORVU840.1_as312		TTGCCTGTGTTTGCATGTGC	20	60.53
HORVU1Hr1G000860.7	**2302070-2309447**	m_c_55033_s941	799	GGATGCAATGCTATTCTCCTGTT	23	59.11
		m_c_51818 _as142		GCACGGCATGTCAGAGTTCA	20	60.95
		m_c_51818 _s66	936	GCAACTCTGACACAGCTGC	19	59.43
		m_c_51818 _as1002		AGATGGGATGCACTCGAAGA	20	58.51
		m_c_51818 _s834	689	GGACAGGAAATGCTGAGGGT	20	59.67
		m_c_51818 _as1523		TCCATCTTGTCCGTTGCGAA	20	59.97
		m_c_51818 _s1293	822	AAGGTGCTTGCAACAAGGGT	20	60.69
		m_c_51818 _as2115		GCCATTGCAGCATCTATCAAAAT	23	58.37

Appendix 13. continued

Gene	Position in 1H	Primer	product size	sequence	bases	tm
HORVU1Hr1G000900.5	**2323680-2330056**	m_c_51837_s2848	881	CCCGTTTCTCATCCAACAAGC	21	59.8
		m_c_51837_as3729		TCGCTCTTGGTTGGGAACTG	20	60.25
		m_c_51837_as5675		GATGGGACGCTGCTAAAGGA	20	59.82
		m_c_51837_s5568	702	CACTGAGTTTTGGTGCTTGAGG	22	59.97
		m_c_51837_as6270		AGATGGAGTAAGGTTGGCGAC	21	59.79
		m_c_51837_s6195	891	GTACCTCCAGAACCTGCAGC	20	60.39
		m_c_51837_as7086		AGTCTTGAAACCACTGGAAAACA	23	58.66
		m_c_51837_s6896	895	TCACTTGGCTTCATGGTCACA	21	59.86
		m_c_51837_as7791		CAGTTGCTGCTTTGGGTGTG	20	60.25
		m_c_51837_s7695		TCTTACTGGGAGATGCTTCAACT	23	59.16
		morex_contig_51837 _ap_as8497		TGTGCTGCGGGAGTTACTTT		
HORVU1Hr1G000910.9	**2362586-2367013**	m_c_54254_s5742	875	GCTCACGGACTAGTCTTGGT	20	59.11
		m_c_54254_as6617		CAATGTCCCGGCATCTCTCA	20	59.82
		m_c_54254_s6598	641	TGAGAGATGCCGGGACATTG	20	59.82
		m_c_54254_as7239		CCCTAAAGGTGCTCAAAATGGAA	23	59.17
		m_c_54254_s7176	849	CCTTCAGTAGCAGCATCCCA	20	59.46
		m_c_54254_as8025		AGGTGCTCGACAATTTCAAACC	22	59.71
		m_c_54254_s7977	712	ATCTTCAGAACCTGCAGCCC	20	60.03
		m_c_54254_as8689		AAGGAACCAAGTCGAACCGA	20	59.25
		m_c_54254_s8568		AGTGTTTGAATGGCAGGAGATTT	23	58.85
		m_c_54254_s9114		CGAAGTACAGATCCGAGTGCA	21	59.87
		m_c_158429_as5744		CCGCCGTGAAGCCAGGA	17	62.15

Appendix 14. Sequence alignment of parental lines and barley reference genome and corresponding SNPs originating from candidate genes located in target interval.

Gene	Alignment position	Type of mutation	Codon	Amino acid substitution	Mutation/SNP	
					Genomic	MBR1012/Scarlett
					Nucleotide	Nucleotide
LC						
HORVU1Hr1G000880	126	E	ATA->ACA	I->T	A	G
	160	E	CCA->TCA	P->S	G	A
	220	E	AAC->CAC	N->H	T	G
	281	E	CGC->CGT	synonymous	G	A
HORVU1Hr1G000970	300	E	GAT->GGT	D->G	T	C
	302	E	CAA->CAG	synonymous	T	C
	303	E	CAA->CGA	Q->R	T	C
	304	E	CAA->GAA	Q->E	G	C
	306	E	GCG->GGG	A->G	G	C
	308	E	GCG->GCA	synonymous	C	T
	309	E	GCG->GGG	A->G	G	C
	328	E	ACC->GCC	T->A	T	C
	345	E	CAA->CAG	synonymous	T	C
	358	E	CGC->CAC	R->H	C	T
	415	E	CGG->CTG	R->L	C	A
	435	E	GCC->GCT	synonymous	G	A
	457	E	CCG->CGG	P->R	G	C
	471	E	TGC->TGT	synonymous	G	A
HORVU1Hr1G001100	1582	E	ACT->AGT	T->S	C	G
	1614	E	GGG->TGG	G->W	G	T
	1643	E	CGT->CGC	synonymous	T	C
	1784	I			C	A
	1816	E	AAT->AAC	synonymous	T	C
	1837	E	GGC->GGT	synonymous	C	T
	1842	E	CCT->CTT	P->L	C	T
HC						
HORVU1Hr1G000830	3415	E	AGT->AAT	S->N	C	T
	3416	E	AGT->GGT	S->G	T	C
	3486	E	CTT->CTA	synonymous	A	T
	3489	E	CAT->CAA	H->Q	A	T
	3533	E	ATA->GTA	I->V	T	C
	3746	E	CTG->GTG	L->V	G	C
	3847	E	ATA->AGA	I->R	A	C
	4262	E	AAC->GAC	N->D	T	C
	4266	E	ATC->ATT	synonymous	G	A
	4303	E	ATT->ACT	I->T	A	G
	4317	E	ATT->ATC	synonymous	A	G
	4332	E	ACC->ACA	synonymous	G	T
	4335	E	CAA->CAC	Q->H	T	G

Appendix 14. continued

Gene	Alignment position	Type of mutation	Codon	Amino acid substitution	Mutation/SNP	
					Genomic	MBR1012/Scarlett
					Nucleotide	Nucleotide
HORVU1Hr1G000830	4336	E	CAA->CGA	Q->R	T	C
	4357	E	CCA->CTA	P->L	G	A
	4362	E	CAG->CAA	synonymous	C	T
	4368	E	TTC->TTG	F->L	G	C
	4375	E	AGT->AAT	S->N	C	T
	4425	E	CTT->CTC	synonymous	A	G
	4428	E	GTT->GTC	synonymous	A	G
	4440	E	GAG->GAA	synonymous	C	T
	4443	E	CTC->CTG	synonymous	G	C
	4450	E	AGG->ATG	R->M	C	A
	4473	E	CAG->CAC	Q->H	C	G
	4486	E	GCT->GTT	A->V	G	A
	4487	E	GCT->CCT	A->P	C	G
	4488	E	CGA->CGT	synonymous	T	A
	4508	E	ATC->CTC	I->L	T	G
	4512	E	ATA->ATT	synonymous	T	A
	4726	E	AAG->ACG	K->T	T	G
	4779	E	TCA->TCC	synonymous	T	G
	4863	U			C	T
HORVU1Hr1G000860	2639	I			T	C
	3351	E	GTG->GTA	synonymous	C	T
	3461	E	CAT->TAT	H->Y	G	A
	3467	E	TTA->ATA	L->I	A	T
	3484	E	GCC->GTC	A->V	G	A
	3524	E	AAG->GAG	K->E	T	C
	3600	E	CTT->CTG	synonymous	A	C
	3675	E	CTT->CTC	synonymous	A	G
	3825	E	CCG->CCA	synonymous	C	T
	3834	E	TCG->TCA	synonymous	C	T
	3848	E	ATT->GTT	I->V	T	C
	3865	E	AAA->ATA	K->I	T	A
	3918	E	TGC->TGT	synonymous	G	A
	3975	E	AAG->AAA	synonymous	C	T
	4010	E	ATT->GTT	I->V	T	C
	4126	E	CCC->CGC	P->R	G	C
HORVU1Hr1G000930	128	E	GGT->GAT	G->D	C	T
HORVU1Hr1G000960	1181	I			G	A
	1201	E	CAA->CAG	synonymous	T	C
	1269	E	GCC->ACC	A->T	C	T

Appendix 14. continued

Gene	Alignment position	Type of mutation	Codon	Amino acid substitution	Mutation/SNP	
					Genomic	MBR1012/Scarlett
					Nucleotide	Nucleotide
HORVU1Hr1G001040	70	E	TGA->TAA	synonymous	C	T
	72	E	AGA->AGC	R->S	T	G
	74	E	AGA->TGA	R->*	T	A
	76	E	GAG->GCG	E->A	T	G
	77	E	GAG->CAG	E->Q	C	G
	82	E	CAC->CCC	H->P	T	G
	83	E	CAC->AAC	H->N	G	T
	84	E	AGC->AGT	synonymous	G	A
	86	E	AGC->TGC	S->C	T	A
	87	E	GGC->GGT	synonymous	G	A
	91	E	GAC->GGC	D->G	T	C
	93	E	TAC->TAT	synonymous	G	A
	98	E	GGC->TGC	G->C	C	A
	99	E	AAC->AAA	N->K	G	T
	102	E	GCA->GCC	synonymous	T	G
	103	E	GCA->GGA	A->G	G	C
	105	E	GAT->GAG	D->E	A	C
	106	E	GAT->GGT	D->G	T	C
	109	E	CAC->CGC	H->R	T	C
	110	E	CAC->TAC	H->Y	G	A
	113	E	AAG->CAG	K->Q	T	G
	114	E	GAC->GAT	synonymous	G	A
	115	E	GAC->GTC	D->V	T	A
	116	E	GAC->AAC	D->N	C	T
	117	E	CAG->CAA	synonymous	C	T
	118	E	CAG->CTG	Q->L	T	A
	119	E	CAG->TAG	Q->*	G	A
	120	E	GTT->GTC	synonymous	A	G
	121	E	GTT->GCT	V->A	A	G
	123	E	CAT->CAC	synonymous	A	G
	124	E	CAT->CCT	H->P	T	G
	125	E	CAT->GAT	H->D	G	C
	126	E	GCC->GCT	synonymous	G	A
	127	E	GCC->GAC	A->D	G	T
	130	E	TTG->TAG	L->*	A	T
	131	E	TTG->ATG	L->M	A	T
	132	E	TGG->TGC	W->C	C	G
	135	E	CAC->CAA	H->Q	G	T
	136	E	CAC->CCC	H->P	T	G
	137	E	CAC->GAC	H->D	G	C

Appendix 14. continued

Gene	Alignment position	Type of mutation	Codon	Amino acid substitution	Mutation/SNP	
					Genomic	MBR1012/Scarlett
					Nucleotide	Nucleotide
HORVU1Hr1G001040	138	E	TGC->TGG	C->W	G	C
	139	E	TGC->TAC	C->Y	C	T
	140	E	TGC->CGC	C->R	A	G
	143	E	GGC->TGC	G->C	C	A
	145	E	TGT->TCT	C->S	C	G
	146	E	TGT->GGT	C->G	A	C
	147	E	TTC->TTT	synonymous	G	A
	148	E	TTC->TCC	F->S	A	G
	149	E	TTC->CTC	F->L	A	G
	150	E	GCA->GCT	synonymous	T	A
	152	E	GCA->CCA	A->P	C	G
	153	E	TTC->TTA	F->L	G	T
	154	E	TTC->TCC	F->S	A	G
	155	E	TTC->ATC	F->I	A	T
	158	E	ATC->GTC	I->V	T	C
	159	E	ACA->ACC	synonymous	T	G
	162	E	TAG->TAC	*->Y	C	G
	163	E	TAG->TGG	*->W	T	C
	166	E	GCC->GTC	A->V	G	A
	167	E	GCC->ACC	A->T	C	T
	170	E	AGC->GGC	S->G	T	C
	171	E	ACA->ACC	synonymous	T	G
	172	E	ACA->AGA	T->R	G	C
	175	E	AAC->AGC	N->S	T	C
	176	E	AAC->TAC	N->Y	T	A
	177	E	TGC->TGA	C->*	G	T
	178	E	TGC->TCC	C->S	C	G
	180	E	AAT->AAA	N->K	A	T
	181	E	AAT->ACT	N->T	T	G
	184	E	GCA->GAA	A->E	G	T
	187	E	GAT->GCT	D->A	T	G
	190	E	GGC->GAC	G->D	C	T
	191	E	GGC->TGC	G->C	C	A
	192	E	GTA->GTC	synonymous	T	G
	193	E	GTA->GCA	V->A	A	G
	196	E	AGC->ATC	S->I	C	A
	199	E	TTC->TGC	F->C	A	C
	202	E	TGT->TTT	C->F	C	A
	203	E	TGT->AGT	C->S	A	T
	205	E	TGA->TCA	*->S	C	G

Appendix 14. continued

Gene	Alignment position	Type of mutation	Codon	Amino acid substitution	Mutation/SNP	
					Genomic	MBR1012/Scarlett
					Nucleotide	Nucleotide
HORVU1Hr1G001040	206	E	TGA->GGA	*->G	A	C
	207	E	GCA->GCT	synonymous	T	A
	208	E	GCA->GTA	A->V	G	A
	211	E	GCT->GGT	A->G	G	C
	212	E	GCT->TCT	A->S	C	A
	216	E	GAT->GAC	synonymous	A	G
	217	E	GAT->GGT	D->G	T	C
	220	E	GCA->GTA	A->V	G	A
	221	E	GCA->CCA	A->P	C	G
	224	E	CGT->TGT	R->C	G	A
	225	E	GAC->GAT	synonymous	G	A
	228	E	TGT->TGA	C->*	A	T
	229	E	TGT->TAT	C->Y	C	T
	230	E	TGT->GGT	C->G	A	C
	231	E	AGG->AGC	R->S	C	G
	233	E	AGG->TGG	R->W	T	A
	234	E	AGG->AGA	synonymous	C	T
	235	E	AGG->AAG	R->K	C	T
	236	E	AGG->GGG	R->G	T	C
	237	E	AGC->AGA	S->R	G	T
	238	E	AGC->ACC	S->T	C	G
	240	E	GCA->GCC	synonymous	T	G
	243	E	CTG->CTA	synonymous	C	T
	244	E	CTG->CCG	L->P	A	G
	245	E	CTG->GTG	L->V	G	C
	246	E	TGC->TGG	C->W	G	C
	247	E	TGC->TTC	C->F	C	A
	248	E	TGC->GGC	C->G	A	C
	251	E	GCA->CCA	A->P	C	G
	252	E	CTT->CTA	synonymous	A	T
	253	E	CTT->CAT	L->H	A	T
	256	E	ATC->AGC	I->S	A	C
	257	E	ATC->GTC	I->V	T	C
	258	E	GGC->GGG	synonymous	G	C
	259	E	GGC->GTC	G->V	C	A
	260	E	GGC->AGC	G->S	C	T
	262	E	GGC->GAC	G->D	C	T
	263	E	GGC->AGC	G->S	C	T
	264	E	TAA->TAG	synonymous	T	C
	265	E	TAA->TTA	*->L	T	A

Appendix 14. continued

Gene	Alignment position	Type of mutation	Codon	Amino acid substitution	Mutation/SNP	
					Genomic	MBR1012/Scarlett
					Nucleotide	Nucleotide
	266	E	TAA->CAA	*->Q	A	G
	267	E	AAT->AAC	synonymous	A	G
	268	E	AAT->ATT	N->I	T	A
	269	E	AAT->GAT	N->D	T	C
	270	E	CTG->CTT	synonymous	C	A
	273	E	GCA->GCG	synonymous	T	C
	274	E	GCA->GTA	A->V	G	A
	277	E	CCC->CTC	P->L	G	A
	278	E	CCC->ACC	P->T	G	T
	280	E	GCC->GGC	A->G	G	C
	281	E	GCC->TCC	A->S	C	A
	283	E	TGT->TTT	C->F	C	A
	284	E	TGT->GGT	C->G	A	C
	285	E	GCA->GCC	synonymous	T	G
	290	E	ATA->GTA	I->V	T	C
	292	E	TCC->TTC	S->F	G	A
	293	E	TCC->GCC	S->A	A	C
	296	E	ACC->GCC	T->A	T	C
HORVU1Hr1G001040	297	E	ACA->ACC	synonymous	T	G
	299	E	ACA->GCA	T->A	T	C
	300	E	GGT->GGG	synonymous	A	C
	301	E	GGT->GTT	G->V	C	A
	306	E	CGG->CGC	synonymous	C	G
	307	E	CGG->CTG	R->L	C	A
	309	E	GCA->GCT	synonymous	T	A
	311	E	GCA->TCA	A->S	C	A
	313	E	TTA->TCA	L->S	A	G
	318	U			G	C
	319	U			G	A
	320	U			A	T
	321	U			C	T
	323	U			G	A
	324	U			C	T
	325	U			T	G
	582	U			A	T
	585	U			T	G
	591	U			C	A
HORVU1Hr1G001060	740	I			A	C
	949	I			G	A

*: Stop codon
E: Exonic
I: Intronic
U: Upstream
D:Downstream

10 Abbreviations

Abbreviation	Meaning
%	Percent
AFLP	Amplified fragment length polymorphism
BAC	Bacterial artificial chromosome
BGS	Barley genome scaffold
BLAST	Basic Local Alignment Search Tool
BOPA	Barley Oligonucleotide Pooled Assay
bp	Base pair (s)
BSA	Bulk segregation analysis
ca	Circa
CAPS	Cleaved amplified polymorphic sequences
cDNA	Complementary DNA
cg	Candidate gene
CH	Chromosome
cM	Centimorgan
cm	Centimeter
CRISPR/Cas	Clustered regularly interspersed short palindromic repeats
CTAB	Cetyltrimethyl Ammonium Bromide
cv	Cultivar
°C	Grad Celsius
DAB	3,3'-diaminobenzidine
dH2O	de-ionized water
ddH2O	Double-distilled water
DArT	Diversity array technology
df	Degree of freedom
DH	Doubled haploid
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
dpi	Days post inoculation
EDTA	Ethylenediaminetetraacetic acid
e.g.	Exempli gratia
etc.	Et cetera
ESTs	Expressed sequence tags
F_1	First fitial generation

FAO	Food and Agriculture Organization of the United Nations
f. sp.	Formae Special
g	Gram
Gb	Gigabases
GBS	Genotyping-by-sequencing
gRNA	Guide RNA
GZ	Genome zipper
h	Hours
ha	Hectare
H. bulbosum	Hordeum bulbosum L.
HCl	Hydrogen chloride
HC	High confidence genes
HMC	Haustorial mother cells
H2O2	Hydrogen peroxide
hpi	Hours post inoculation
HPLC	High performance liquid chromatography
HR	Hypersensitive reaction
HRMP	High-resolution mapping population
HT	High throughput sequencing technology
HTP	High-throughput
H. spontaneum	*Hordeum vulgare* ssp. *spontaneum*
H. vulgare	*Hordeum vulgare* L. ssp. *vulgare*
i.e.	Id est
IBSC	International Barley Genome Sequencing Consortium
IT	Infection type
KASP	Kompetitive Allele Specific PCR
kb	kilobase
kg	kilogram
LC	Low confidence genes
LD	Linkage disequilibrium
LLR	leucine rich repeat
LOD	Logarithm od the odd
Mb	Megabase pairs
M	Molar
MAS	Marker-assisted selection
Mg	Milligram

MgCl2	Magnesium chloride
min	Minute
µg	Microgram
µl	Microliter
ml	Millilitter
µm	Micrometer
NBS	Nucleotide binding sites
ng	Nanogram
NGS	Next-Generation Sequencing
nm	Nanomiter
Pa	*Puccinia anomala*
pH	Negative decimal logarithm of H+ concentration
P. hordei	*Puccinia hordei* Otth
PCR	Polymerase chain reaction
pmol	Picomole
POPSEQ	Population sequencing
QBS	Quedlinburg barley SNP
QTL	Quantitative trait loci
R	Resistance
RAPDs	Random amplified polymorphic DNAs
Ren-seq	R gene enrichment sequencing
RFLPs	Restriction fragment length polymorphisms
RILs	Recombinant inbred lines
RNA	Ribonucleic acid
RNAi	RNA interference
ROS	reactive oxygen species
Rph	Resistance against *P. hordei*
g	times gravity
RT	Room temperature
RT-PCR	Real-time PCR
s	Second
Seq	Sequence
SNPs	Single nucleotide polymorphisms
sp	Single species
STS	Sequence Tagged Site
SSR	Simple sequence repeats

t	Ton
Taq	Thermophilus aquaticus
TALEN	Transcription activator-like effector nucleases
TBE	Tris/Borate/EDTA
Tris	2-Amino-2-hydroxymethyl-propane-1,3-diol
U	Unit
UV	Ultraviolet
WGS	Whole-genome shotgun assembly
w/v	Weight per Volume
w/w	Weight per Weight
V	Volt
VIGS	Virus induced gene silencing
v/v	Volume per Volume
YAC	Yeast Artificial Chromosome

11 List of figures

12 List of tables

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Declaration under oath

Eidesstattliche Erklärung / Declaration under Oath

Ich erkläre an Eides statt, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

I declare under penalty of perjury that this thesis is my own work entirely and has been written without any help from other people. I used only the sources mentioned and included all the citations correctly both in word and content.

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