Nonhost resistance to rusts (*Puccina* sp.) in Barley (*Hordeum vulgare* L.)

Fine mapping project

Daan Bomhoff

2014

MSc. Major Thesis (PBR-30806)

PLANT BREEDING
WAGENINGEN UR

WAGENINGEN UR

Nonhost resistance to rusts (*Puccina* sp.) in Barley (*Hordeum vulgare* L.)

Fine mapping project

Daan Bomhoff

2014

MSc. Major Thesis (PBR-30806)

A thesis submitted in partial fulfillment of the Master of Science degree in Plant Sciences
Specialization Plant Breeding and Genetic Resources

Supervisors:
Dr. Rients E. Niks[1]
Dr. Alberto Martín-Sanz[1,2]

Examiners:
Dr. Rients E. Niks[1]
Dr. M.J.W. Jeuken[1]

WUR registration number: 890924093020
Date of submission: 20-3-2014
E-mail: daan.bomhoff@wur.nl

Keywords: Barley, *Hordeum vulgare*, fine mapping, L94, Nonhost resistance, Plant resistance, *Puccinia*, *Rnhq121cM* , *Rphq11*, Rusts, SusPtrit, SusQ11

[1] Department of Plant Breeding, Wageningen University, P.O. Box 386, 6700 AJ Wageningen, the Netherlands.
[2] Pioneer Hi-Bred Spain S.L., Avda del Reino Unido nº 7, 2ª planta, 41012, Sevilla, Spain.

PLANT BREEDING WAGENINGEN UR

Front picture: *Puccinia hordei* on Barley.
Taken from http://www.scienceinpublic.com.au/media-releases/barley-rust on 17-3-2014.

Table of Contents

Acknowledgments

This MSc thesis would not be completed without the help, advice, criticism and spirit of teachers, friends and family. Therefore, I would like to dedicate this small section of my report to them.

This MSc thesis would not have come to fruition without the support, knowledge and unwavering enthusiasm of my supervisor Dr. Rients E. Niks. I'm grateful for all the talks we had and the criticism you showed when examining my work. You're a very knowledgeable researcher in the field of plant breeding that knows how to get the point across.
Secondly, I'm very grateful of the supervision of Dr. Alberto Martin Sanz. His knowledge of the finer details of the project and his skill in the molecular lab helped me immensely during the first phases of this study. Sorry again for forgetting to turn on the humidity chamber, when it was already past the opening hours of Unifarm. Good luck in Spain!
Thirdly, I would like to thank Anton Vels. Things would have gone much slower, or not at all, without your help and practical knowledge. Also, I really liked the talks we had on various topics and especially those about practical plant breeding. You and Rients make a very good team.
I would also like to thank Koen Pelgrom, with his help understanding various parts of my thesis. You made sure I did not sequence all of those PCR products! Which I, and probably Rients as well, am very grateful of.
I would also like to thank Cynara Romero. As both students, we kept each other motivated and up-to-date during the work. Thanks to you I'm a plant breeder now!
I'm indebted to Abhaysinh Salunke, for providing me with the necessary data to finish this thesis. I think your motivation will allow you to become a skilled researcher.
Appreciation goes to all the lab technicians, Johan Bucher, Marjan Oortwijn and Gert van Arkel who helped during the molecular work of my thesis. Your practical knowledge is essential for almost every student at the plant breeding department. I really liked the talks we had in the train Johan; I'm jealous of your spirit and dedication.
I would also like to thank Betty Henken, with helping me finding my way around the lab when I was in the process of staining the leaves. I hope you liked the pictures.
I would also like to thank Marieke Jeuken, for being my second examiner.

And last but not least, I would like to thank my friends, family and especially my girlfriend Vivian Wilschut, for their patience with me. Vivian, you are the world to me. For taking care of me after the long hours of work and putting up with my moments of stress. I know that I should learn how to plan ahead more efficiently.

Thank you department of Plant Breeding of the Wageningen University and Research Centre!

Summary

The identification of genes conferring nonhost resistance (NHR) is a crucial step in its understanding and usage in breeding. The Barley(*Hordeum vulgare* L.)-*Puccinia* (rust fungi) model system uses marginal hosts of barley to *Puccinia* rusts to study NHR, making the identification of NHR genes possible. This study entails the fine mapping work of two NHR genes; one located on the introgression of the NIL SusQ11, near *Rphq11*, and the other located on L94.

The NIL SusQ11 (Steptoe x SusPtrit) and subsequent homozygous subNILs (SusQ11 x SusPtrit) were initially constructed to (fine) map *Rphq11*, a QTL of Steptoe found to confer partial resistance to *Puccinia hordei* (*Ph*). However, SusQ11 also proved to be higher resistant to heterologous rusts. Studies indicated that on the region between 121-128cM of the introgression resistance to the heterologous pathogens *Puccinia-hordei secalini* (*Phs*) and *Puccinia-hordei bulbosi* (*Phb*) was located. In addition, resistance to the heterologous rust *Puccinia persistens* (*Pp*) was mapped between 120-121cM. In this study, the subNILs were genotyped and phenotyped using newly developed markers of the 121-128cM region and inoculation with the heterologous rusts *Pp*, *Phb* (Israël isolate), *Phs*, *Puccinia-hordei murini* (*Phm*), *Puccinia triticina* (*Pt*) and *Puccinia graminis* f. sp. *lolii* (*Pgl*) respectively. The developed markers were not able to fine map the 121-128cM region. QTL mapping and substitution mapping was performed and showed an association of *Pp*, *Phs*, *Phb_Isr*, *Phb_Iran*, *Pt* and *Phm* with the markers in the 121-128cM region (hereinafter called *Rnhq121cM*). *Pgl* was only associated with *Rnhq121cM* by means of the QTL analysis. Earlier studies indicated that a two locus interaction exists for the pathogen *Phb_Iran*, between *Rphq11* and *Rnhq121cM*. This interaction was assessed histologically and results suggested a high effect of *Rnhq121cM* on the percentage of early abortions and width of infections. A smaller effect of *Rphq11*, on the speed of sporulation, was also found. No two locus interaction was found for the other rusts tested in this study.

Studies showed a qualitative resistance of the heterologous rusts *Pp* and *Phb_Isr* in the Ethiopian barley line L94. The cross L94 x SusPtrit showed a more quantitative form of this resistance. BSA identified three markers associated with resistance to *Pp* at 2H:132.3, 2H:123.3 and 6H:60.6 (marker 3, 8, 26) and two markers associated with *Phb_Isr* resistance at 2H:132.3 and 5H:10.6 (marker 8 and 13). The markers of *Pp* resistance explained most of the phenotypic variation. In this study, plants segregating for these markers (except marker 13) were genotyped with the markers 3, 8, 26 and the additional marker 36(6H:60.6). Phenotyping with *Pp* and *Phb_Isr* (partly in this study) was performed for these plants. In the end, marker 3 and 26 were genotyped in the segregating material; the rest of the markers failed to produce polymorphic results. These markers showed an association with *Pp* and not *Phb_Isr,* as found previously. The phenotypic variation of *Pp* was only partly explained.

Results of this study are a step towards the fine mapping of NHR resistance genes.

1. Introduction

The focus of this thesis is fine mapping of genes related to non-host resistance to rusts (*Puccinia* sp.) in Barley (*Hordeum vulgare* L.). In this report, the underlying basic principles of plant defense will be described first; later a detailed research background will be given.

1.1 Basic concepts of plant defense

Plants are constantly subjected to stress factors. There are countless potential sources of these stress factors, however a large part is caused by plant pathogens; micro-organisms which live inside the plant and cause disease and their subsequent symptoms. Several types of plant pathogens are characterized on the basis of their life-style such as biotrophic, necrotrophic and hemibiotrophic pathogens. *Puccinia* rusts (Basidiomycota, Uredinales, Pucciniaceae) are an example of obligate biotrophic fungi. These obligate fungi form haustoria inside plants cells, which actively extract nutrients from living cells (Figure 1). *Puccinia* rusts are either autoecious or heteroecious.

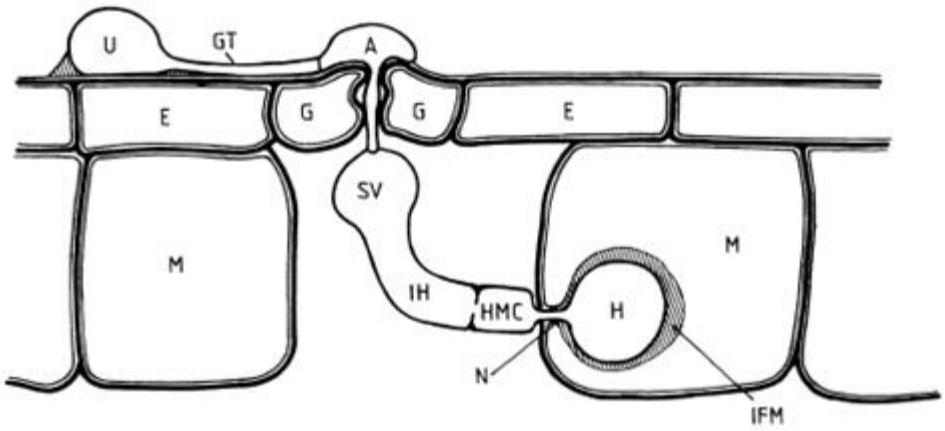


Figure 1. Schematic illustrating the generalised infection process of a rust fungus in the dykariotic stage. Uredospores (U) germinate to form germ tubes (GT) which sense the topography of the host cuticle (thigmotrophism) and develop appressoria (A) above stomata (guard cells = G). The GT then penetrates through stomata into substomatal spaces where the fungus differentiates substomatal vesicles (SV). From these SV, intercellular hyphae form (IH) which come into contact with host mesophyll cells (M) and develop haustorial mother cells (HMC). Following this, haustorial formation is initiated, neckbands (N) are formed around the site of penetration of the mesophyll cell and an interfacial matrix (IFM) develops between the haustoria cell wall and host cell plasma membrane. After a period of successful feeding, the rust develops new spores and a pustule is formed. Adapted from Perfect and Green (2001).

Plants cannot move to evade these pathogens; they have therefore evolved a two branched innate immune system (Bent and Mackey, 2007). The first branch recognizes and responds to molecules conserved across many classes of microbes, the second responds to pathogen effectors, either directly or through their effects on host targets (Jones and Dangl, 2006).

Although there are three basic defense strategies in plants to pathogens (avoidance, tolerance and resistance) (Niks et al., 2011) the focus of this thesis is mainly on plant resistance. Plants can defend themselves against pathogens by a constitutive (wax layers, secondary metabolites etc.) and an induced (PR-proteins, papillae etc.) defense. Induced defense is trigged by the recognition of PAMPs

(Pathogen-Associated Molecular Patterns), compounds or peptides of the pathogen which are essential for the functioning of the pathogen (for example fungal chitin). PAMPs are conserved among pathogen species (Nuernberger and Lipka, 2005). Recognition of a PAMP may lead to PAMP-Trigged Immunity (PTI) (Bent and Mackey, 2007; Niks et al., 2011). Together with the constitutive defense response, PTI is considered to be a general form of defense and is effective against a broad range of pathogens (Niks et al., 2011).

If a pathogen is able to negate the general defense response and able to exploit the plant species, this is called basic compatibility. The plant is then a host of the pathogen. In order to negate the plant defense, the pathogen can excrete specific proteins, called effectors. These effectors suppress the PTI and lead to Effector-Triggered Susceptibility (ETS). In turn, the plant has evolved R-genes which recognize the products of avirulence (*Avr*) genes of the pathogens and lead to a qualitative form of resistance which is called Effector-Triggered Immunity (ETI). It is often associated with a hypersensitive response (see §1.2). An overview of the different defense responses is given in figure 2.

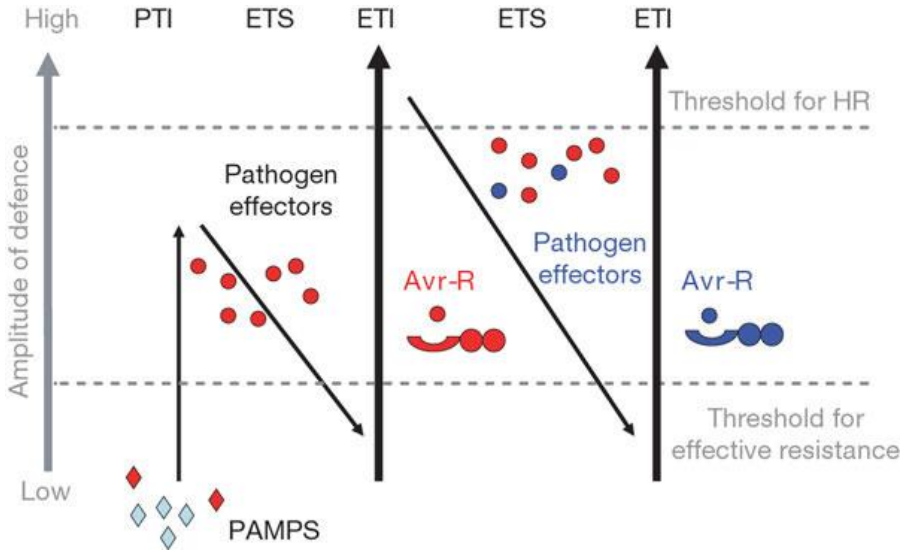


Figure 2. Schematic "zig-zag" overview of plant defense. The plant is able to recognize the pathogen, leading to immunity. However, the pathogen is able to evade or suppress the defense response by secreting effectors (Jones and Dangl, 2006).

1.2 (Non-)Host resistance

Generally, genotypes within a plant species are unequally susceptible/resistant to a pathogen. The pathogen is then able to suppress/avoid the PTI and other (constitutive) defense responses (basic compatibility). However, certain genotypes of the plant species have evolved an (extra) layer of resistance which is called host resistance (Bent and Mackey, 2007; Marcel, 2007; Niks et al., 2011). Host resistance can be categorized in two types of resistance, namely (i) partial resistance and (ii) the Hypersensitive Response (HR) (Niks et al., 2011). Partial resistance or horizontal resistance is when the infection by the pathogen is reduced despite a compatible interaction between plant and pathogen. Partial resistance is a quantitative form of resistance which is controlled by Quantitative Trait Loci's (QTLs) on the plant genome. It functions pre-haustorially and it is presumed that a large part functions on the basis of a basal PTI response that is only partly suppressed (Niks and Marcel,

2009). As partial resistance is polygenetically inherited, it is a durable form of resistance (Parlevliet and Zadoks, 1977; Yeo, 2007).
The HR or vertical resistance involves the active necrosis by the plant of the plant its own infected cells (Programmed Cell Death) to arrest the infection by the pathogen. The HR occurs after the formation of a haustorium. The HR has a qualitative nature and is governed by major genes (R genes) in contrast to the QTLs of partial resistance. R genes lie in clusters of functional and non-functional homologues, with different alleles (Bent and Mackey, 2007; Niks et al., 2011). These R genes function on a gene-for-gene basis (Flor, 1971; Niks et al., 2011), where a resistance gene is only effective if the infecting pathogen carries the corresponding *Avr* gene. While the R genes provide a high level of resistance, the effectiveness in breeding is limited due to a low durability (den Boer et al., 2013). Resistance can easily be overcome by, for example, loss-of-function mutations in *Avr* genes in the pathogen (Chen et al., 2010). This is especially the case in rapid changing pathogen populations, for example in big, sexual reproducing, populations with a high selection pressure and high gene flow (McDonald and Linde, 2002). Pathogens that are generally able to infect a certain plant species are called homologous pathogens. An example of this is *Puccinia hordei* (*Ph*) in barley.

However, disease is the exception, not the rule in nature. Plants are often (fully) resistant against infection by most of the pathogen species (Atienza et al., 2004). If a plant species is immune against all genotypes of a pathogen species, this type of resistance is called non-host resistance (NHR) (Heath, 2000). Unadapted pathogens are then not able to establish basic compatibly with the plant. NHR resistance results from a continuum of layered defense responses, entailing both constitutive and induced defense mechanisms, resulting in a complete form of basal resistance (Lipka et al., 2008; Niks et al., 2011; Nuernberger and Lipka, 2005). Induced NHR starts before the formation of a haustorium and leads to the formation of papillae, but some instances of hypersensitive post-penetration resistance have been reported (Jafary et al., 2006a; Jafary et al., 2008; Niks and Marcel, 2009). It is speculated that NHR functions on the basis of the PTI mechanism (Niks and Marcel, 2009; Nuernberger and Lipka, 2005). There has been no indication that the HR response mechanism is identical to the one observed in HR (R-avr interaction) (Niks and Marcel, 2009). Pathogens that are unadapted or maladapted to a non-host plant species are called heterologous pathogens.
Non-host resistance is observed as complete, durable and occurring in most of the crop species, but for a large period of time it has not been considered as a major source of resistance for breeding practices (Hammond-Kosack and Parker, 2003). The most important reason for this are the barriers between species that prevent genetic transfer (Johnston et al., 2013). By definition all individuals of the nonhost plant species are completely resistant to the pathogen. The resistance thus has its usefulness in transferring it to other (host) plant species. Therefore, the trait first needs genetic dissection with molecular tools, for example with molecular markers. It is necessary to be able to specify and target key NHR genes or loci for (future) targeted breeding efforts in species other than the nonhost. Markers are needed that are as close to the gene(s) as possible; to reduce the effect of linkage drag. This method has recently demonstrated to be effective, making NHR more and more applicable for breeding practices (Johnston et al., 2013). The effectiveness of NHR breeding has also been demonstrated in breeding for resistance against the pathogen *Bremia lactucae*, as stacking led to increased resistance in Backcross Inbred Lines (BIL) resulting from the cross between the non-host *Lactuca saligna* and susceptible *L. sativa* (den Boer et al., 2013). This thesis details a fine mapping project which attempts to find specific NHR genes for resistance to rusts (*Puccini*a sp.) in the model crop Barley.

1.3 Barley-*Puccinia* model system

As NHR is a species characteristic, as mentioned above, it is inherently difficult to perform inheritance studies to find the position of the genes responsible for the contrast host versus nonhost. Occasionally, it reported that a non-host is crossable with a host species, for example in lettuce (Jeuken, 2002). But in most of the cases, interspecific crosses are needed that are hard, resulting at best in rare progeny that are stunted in growth, sterile and suffer from abnormal segregation (Hammond-Kosack and Parker, 2003, Atienza et al., 2004). Barley is an excellent model species for NHR studies as it is a species that is intermediate between host and non-host status to some rust fungi (Figure 3). There are some rare genotypes that are susceptible to heterologous rusts (also called near-nonhost or marginal hosts). Assuming the genes underlying resistance to near-nonhost resistance are similar to those underlying NHR, these rare individuals can be used to perform inheritance studies (Atienza et al., 2004; Niks, 1988; Niks and Marcel, 2009). By crossing susceptible genotypes and selecting for increased susceptibility, an experimental line named SusPtrit with extreme susceptibility to several heterologous *Puccinia* rusts was created at the department of Plant Breeding at the Wageningen University and Research centre (WUR), a process which is described by Atienza et al. (2004). SusPtrit is often used as susceptible parent in the inheritance studies.

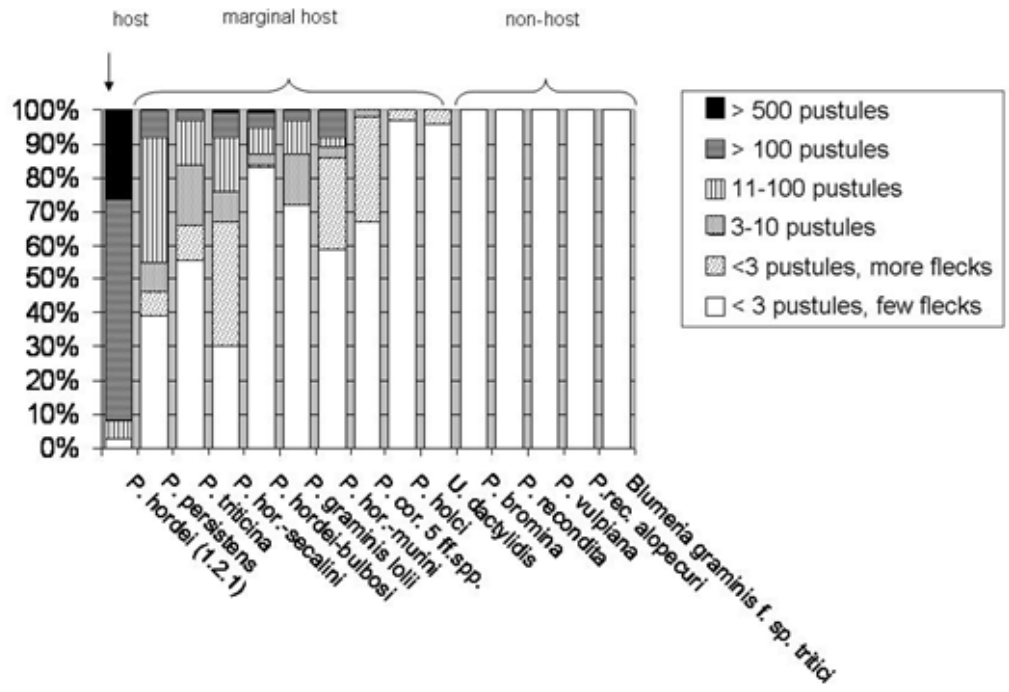


Figure 3. Host status of barley to 14 rust species and one powdery mildew species. The percentage of barley accessions (y-axis, n=110) per susceptibility class (legend) was determined at the seedling stage as described in the methods section. Susceptibility class is measured in number of pustules and flecks on the first seedling leaf. Picture taken from (Niks et al., 2011). Host indicates that almost all of the barley accessions display a high susceptibility, marginal host indicates that a few accessions display a certain form of susceptibility and non-hosts are pathogens that display no infection on any barley accession.

This Barley-*Puccinia* model system was then used to develop mapping populations between regular resistant barley lines and SusPtrit that segregated for resistance to multiple heterologous rusts (for example: *Puccinia persistens* (*Pp*), *Puccinia hordei-bulbosi* (*Phb*), *Puccinia hordei-secalini* (*Phs), Puccinia hordei-murini* (*Phm*), *Puccinia triticina* (*Pt*), *Puccinia graminis* f. sp. *lolii* (*Pgl*)) (Jafary et al.,

2006a; Jafary et al., 2008). With those mapping populations, researchers were able to identify genes that contribute to the defense against heterologous rusts. An example of this are: *Rphq11* (Marcel et al., 2007; Qi et al., 2000, Yeo et al., unpublished) and *Rnhq* (Niks et al., 2000). Some of those genes also affect the resistance level to *Ph* (see §1.4). Important findings were:

(i) The complete resistance in the resistant lines to the heterologous rusts is a phenotypically "qualitative" character with a mostly quantitative inheritance (Jafary et al., 2008).
(ii) NHR is controlled by "sets" of quantitative resistance genes with different and overlapping specificities (Jafary et al., 2006a).
(iii) Plant genotypes (e.g. Vada, L94 etc.) appear to have different (rust specific) NHR loci (Jafary et al., 2006a; Jafary et al., 2008; Marcel et al., 2007; Niks et al., 2000; Qi et al., 1998; Qi et al., 2000).

Detailed fine mapping with molecular markers can elucidate the exact position of these resistance genes and their flanking markers. Fine mapping is then followed by physical mapping and map-based cloning, completing the forward genetics approach (Peters et al., 2003; Remington et al., 2001). During this MSc thesis project a fine mapping procedure of a NHR introgression is continued and a mapping procedure on a relatively new resistance gene (locus) of L94 is continued. The specific projects will be detailed below.

1.4 SusQ11

SusQ11 is a Near-Isogenic Line (NIL) constructed to contain the resistance QTL *Rphq11* in SusPtrit background. *Rhq11* is a QTL that was found to confer partial resistance to the homologous rust *Puccinia hordei* (Qi et al., 2000) and was found in several mapping populations, namely 116-5 x L94 (Qi et al., 2000), Cebada Capa x SusPtrit (Jafary et al., 2006b) and Steptoe x Morex (Marcel et al., 2007b). The QTL of Steptoe was introduced into SusPtrit background by repeated backcrossing. The resulting NIL containing the introgression with *Rphq11*, SusQ11, was surprisingly also quantitatively resistant to a large number of heterologous rusts, namely *Phs*, *Pt*, *Pgl*, *Phm*, *Pp, Phb_Isr* and even almost completely resistant to *Phb_Iran* (Yeo et al., unpublished). After (fine) mapping with Amplified Fragment Length Polymorphism (AFLP) and Single Nucleotide Polymorphism (SNP) markers on sub-NIL homozygous recombinants (from here on called homorecombinants) it was found that the *Rphq11* QTL is located at 91cM (peak marker WBE144 (Kuijken, 2009)) on chromosome 2H of barley (Yeo et al., unpublished) on the barley consensus map of the research group (Martín-Sanz et al., 2012, unpublished). In addition, it was found with markers that the *Rphq11* marker interval conferring partial resistance to *Ph* does not explain all the variation in terms of the observed level of resistance to the heterologous rusts. New research was conducted and markers were developed to fine map the other regions of the introgression of SusQ11, looking for candidate regions for resistance against heterologous rusts described previously. This research showed that the region between 121-128cM (peak marker at 121.05cM) and 120-121cM explain resistance to the rusts *Phs/Phb* and *Pp* respectively (Salunke, 2013). Moreover, a two locus interaction was found between *Rphq11* and the 121-128cM region (called *Phs*/*Phb* QTL hereinafter) (Table 1).

Table 1. Phenotypic data (relative infection frequency with SusPtrit) comparing the different allelic configurations of the QTL *Rphq11* and the found QTL for resistance to the heterologous rusts *Phs*, *Pt*, *Phb_Isr*, *Phb_Iran*, *Pp*, *Phm* and *Pgl* (for *Pp* this QTL is located between 120-121cM; for the rest the QTL is located between 121-128cM). Allele notations: A: Steptoe (resistance); B: SusPtrit (susceptible). Figure and data taken from Salunke (2013). Colors indicate highest and lowest value, from dark green (lowest) to dark red (highest).

Rphq11	*Resistance QTL*	Phs	Pt	Phb ISR	Phb IRAN	Pp	Phm	Pgl
A	A	19.6	37.2	14.1	4.5	17.0	35.9	34.4
B	A	19.7	36.5	11.8	30.7	20.1	40.0	39.1
A	B	84.8	76.8	44.5	59.7	137.6	79.4	117.0
B	B	59.3	70.8	55.1	101.4	181.9	92.3	81.2

The phenotypic data of Salunke (2013) for the different pathogens showed clearly that for the pathogen *Phb_Iran* distinct levels can be found that are influenced by the interaction between the alleles of *Rphq11* and the alleles of 120-121cM (*Pp*) and 121-128cM (rest of the rusts) region. This interaction was already speculated by Yeo et al. (unpublished), when an almost complete resistance was found of SusQ11 to *Phb_Iran*. The rest of the rusts showed no distinct interaction effect.

This MSc thesis continued the work performed by Niks, R.E., Salunke (2013) and many others at the department of Plant Breeding of the WUR, by:
(i) phenotyping the sub-NIL homozygous recombinants (21 recombinants), SusQ11, Steptoe and SusPtrit with the heterologous rusts *Phs*, *Pp*, *Pgl*, *Pt*, *Phm* and *Phb_Isr*. These replicates will be added to a first replicate done by Salunke (2013), in order to improve the reliability of the phenotypic data for the fine mapping. *Phb_Iran* is not used in this test, as a sufficient amount replicates are already performed to achieve reliable results.
(ii) fine map the *Phs*/*Phb* QTL (121-128cM) in the introgression in SusQ11 by developing and running new SNP markers for that region
(iii) determining which markers (newly developed markers and markers already available) are associated with the NHR to *Phs*, *Pp*, *Pgl*, *Pt*, *Phm* and *Phb_Isr* in SusQ1. This is done via a QTL analysis and substitution mapping approach (Marcel et al., 2007a) using the marker data of this and previous studies and the phenotypic data of this and previous studies.
(iv) constructing markers, suitable for KBioscience Competitive Allele-Specific Polymerase chain reaction (KASPar), that flank the *Rphq11* and *Phs/Phb* regions.
(v) performing a histological study, where the mode of resistance to *Phb_Iran* is studied in detail.
(vi) identify whether a two locus interaction exists between *Rphq11* and the region associated with resistance to the other heterologous rusts tested in this study. In addition, identifying, when multiple regions are found conferring resistance to heterologous rusts, if there is interaction between those.

1.5 Resistance to *Pp* and *Phb_Isr* in L94 background

These loci were found in a cross between SusPtrit and L94. L94 is an Ethiopian barley line and is extremely susceptible to *Ph* and also somewhat susceptible to *Pt* and other heterologous rusts (Chisenga, 2013). L94 was, before the development of SusPtrit, often used as a susceptible parent to investigate aspects of NHR (Chisenga, 2013; Niks, 1983a; Niks, 1983b; Qi et al., 1999). Contrastingly, in an experiment, L94 seemed to be almost immune against *Pp* and *Phb_Isr* (Atienza et al., 2004; Chisenga, 2013; Jafary, 2006). A trial experiment was performed by Rients Niks at the department of Plant Breeding of the WUR, where the F_2 of the cross between L94 and SusPtrit showed segregation

for resistance to *Pp* and *Phb_Isr*. This resistance appeared to segregate qualitatively. Bulked Segregant analysis (BSA) on a BC3-S2 generation of the cross identified markers associated with resistance (Rients et al., unpublished). Later, progeny of this material was genotyped with some of the interesting markers and phenotyped with *Pp* and *Phb_Isr* to do a preliminary mapping of the resistance (Chisenga, 2013).
At that time, however, the segregation appeared to be more quantitative. For *Pp* resistance, in total 3 markers on 3 different locations were found to be associated with resistance (2H:123.3cM, 2H:132.3cM and 6H:60.6cM), for *Phb_Isr* resistance 2 markers on 2 different locations (2H:132.3cM, 5H:10.6cM) were found. These markers were called 3, 8 & 26 and 8 & 13 respectively. The QTL in chromosome 2H:132.2cM (marker 8) was common for both pathogens, and was not described previously. The other two resistance QTLs are mapped in regions in which resistance genes to *Pp* are mapped in other mapping populations. The three QTLs for resistance against *Pp* had a dominant effect and explained almost all the phenotypic variability, where the QTL at 123.3 explained most of the variation. In the case of *Phb_Isr*, the main QTL for resistance was the located at 2H:132cM while the one at 5H:10.6cM worked in cooperation with the first one. The QTL at 5H was not mapped previously. For *Phb_Isr*, not all the phenotypic variability was explained, suggesting that more QTLs can be involved in the resistance.

In the next step, plants still heterozygous for those four specific markers were selfed. The progenies of these lines were phenotyped and used in this study to corroborate the association of the mentioned markers with the resistance to *Pp* and *Phb_Isr*.

This MSc thesis continued the work done by Rients Niks, Chisenga (2013) and others at the department of Plant Breeding of the WUR, by:
(i) phenotyping the 1501-2 to 1501-5 progeny, by inoculation with *Pp* and *Phb_Isr.*

(ii) running markers (3, 7, 8, 13, 25, 26 and 36) on the DNA of the 1501-2 t/m 1501-5 progenies to identify plants which can be used in new rounds of recombinant screening. Marker 36 is located on the same location as marker 26.

(iii) confirming/finding an association of the above mentioned markers and the resistance to *Pp* and *Phb_Isr* in the 1501-2 to 1501-5 progenies and determining whether all the phenotypic variation is explained.

1.6 Research questions and objectives

One of the objectives of this thesis was to fine map the resistance to the heterologous rusts *Phs*, *Phb_Isr*, *Pp*, *Pt*, *Pgl* and *Phm* in SusQ11 background. This fine mapping is assisted by more rounds of phenotyping.

The second objective was to genotype/phenotype the 1501-2 t/m 1501-5 progenies to identify whether (i) the association of the four markers with resistance to *Pp* and *Phb_Isr* could be established again and (ii) whether heterozygous progeny for (future) recombinant screenings could be identified.

Research questions *Rphq11*

Q_1. Using more markers and the same homozygous recombinants, is it possible to fine map the resistance to *Phs*/*Phb_Isr* and *Pp* more precisely at the 121-128cM interval?

Q_2. Do the extra replications of the phenotypic tests corroborate the association between the phenotype and the markers of the locus 121-128cM?

Q_3. Are any new associations found between markers and the heterologous rusts *Pgl*, *Phm* and *Pt*, using the (new) phenotypic and genotypic data of the homorecombinants?

Q_4. Is there an interaction between the different resistance loci for heterologous rusts *Phs*, *Pp*, *Pgl*, *Pt_S*, *Phm_R, Phb_Iran* and *Phb_Isr* and is there interaction between *Rphq11* and those resistance loci; assuming that the resistance QTLs can be mapped using the data from this study.

Q_5. Using the new phenotypic data, are there any new regions found that confer resistance to the heterologous pathogens in SusQ11?

Q_6. What is the histological effect of two locus interaction on *Phb_Iran*?

Research questions Resistance gene L94

Q_1. Using the markers from the study done by Chisenga (2013), is it possible to genotype the individuals in the progeny of 1501-2 to 1501-5? And can this information be used to identify individuals for (future) recombinant screenings?

Q_2. Are these markers associated with resistance to *Pp* and *Phb_Isr* in the progeny of 1501-2 to 1501-5?

Q_3. Do these genotyped markers explain all of the phenotypic variation for *Pp* and *Phb_Isr* resistance in the progeny of 1501-2 to 1501-5?

2. Methods

2.1 SusQ11

The NIL SusQ11 was developed by Yeo et al. (data not published) at the department of Plant Breeding by introgressing the resistance QTL of the barley cultivar Steptoe in SusPtrit background by repeated backcrossing (Figure 4). Recombinants were produced by crossing SusQ11 with SusPtrit. These recombinants were selfed in order to produce the sub-NIL homorecombinants with different fragments of the Steptoe genome. This allowed substitution mapping (Marcel et al., 2007a) of the introgression from Steptoe. Selections were performed with the help of markers and by visual phenotyping as described below.

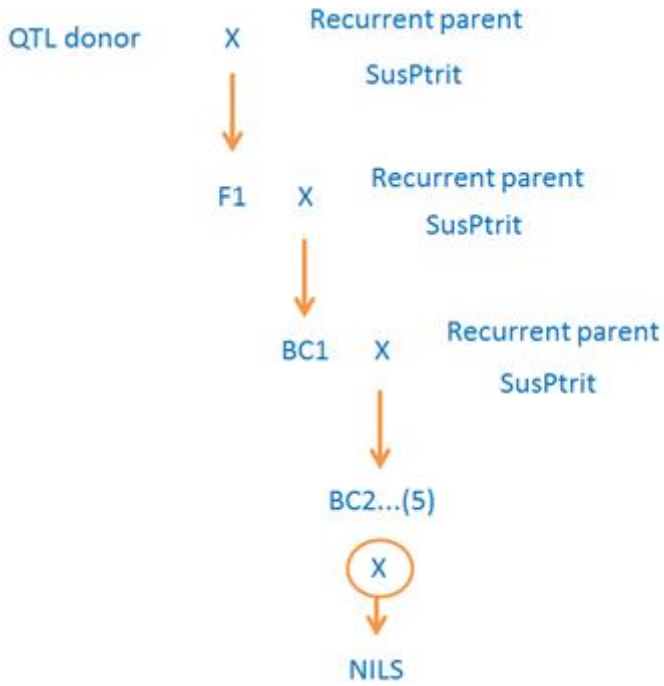


Figure 4. Schematic representation of the development of the homozygous recombinants (homorecombinants) of SusQ11. The QTL donor is SusQ11 and the recurrent parent is SusPtrit. This scheme can also represent the construction of SusQ11 by using as QTL donor Steptoe.

2.1.1 Phenotyping

Seeds were available of SusQ11, the 20 homorecombinants of SusQ11 and of the parents Steptoe and SusPtrit.

Seeds were sown in two rows, in boxes of 37x39cm, along the longest side of the box (Figure 5). Sowing occurred 7 - 10 days prior to inoculation with the rust spores. Three seeds per genotype (Steptoe, SusPtrit, homorecombinant 1 etc.) were sown as at least two individuals were needed for the inoculation. In case of poor emergence, a back-up plant was then available. In total, all of the 24 genotypes (20 homorecombinants + Steptoe, SusPtrit and SusQ11) were sown per box, with a total of 48 plants used in the inoculation. One box was inoculated with one rust species.

The plants were inoculated when the primary leaf was full grown and the secondary leaves started to appear. Prior to the inoculation, the seedlings were bent over so that they rested flat on the soil with their adaxial side up (Figure 5). The primary leaf was kept in that position by multiple iron staples along the length of the leaf. Secondary leaves were clipped. A glass slide was added to each

box to estimate the amount of germinated spores under the light microscope the day after the inoculation.

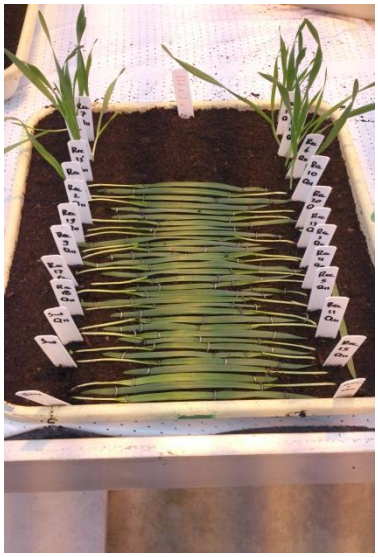


Figure 5. Barley seedlings in a 37x39 box, sown in two rows. Prior to inoculation, seedlings were bent over and hold into place with iron clips.

Inoculum

Six heterologous rusts were used in the phenotyping experiments, as previous research indicated that SusQ11 was quantitatively resistant to these rusts. The respective rusts are *Phs*, *Pp*, *Pgl*, *Pt*, *Phm* and *Phb_Isr*. As *Puccinia* rusts are obligate biotrophs, the rusts were multiplied on their respective hosts (Table 2).

Table 2. Overview of the rusts species used in this study, with their respective natural host. The abbreviation used in this study is given between the parentheses. Host plant data is obtained from Atienza et al. (2004) and freely available internet sources.

Rust	Host plant	Alternate host[a]
Puccinia persistens (*Pp*)	*Agropyron repens*	
Puccinia hordei-secalini – French isolate (*Phs*)	*Hordeum secalinum*	
Puccinia hordei-bulbosi – Israel isolate (*Phb_Isr*)	*Hordeum bulbosum*	
Puccinia triticina – Swiss isolate (*Pt*)	*Triticum aestivum* (genotype 8860)	*Thalictrum sp.*
Puccinia graminis f. sp. *lolii* (*Pgl*)	*Lolium perenne*	*Berberis vulgaris* L.
Puccinia hordei-murini – Rhenen isolate (*Phm*)	*Hordeum murinum*	

a In the case of multiple alternate hosts, the most important alternate host is given.

Uredospores were collected 2h to 3 days prior to inoculation, with a cyclone spore collector. Per box 2.5mg uredospores was used for inoculation. The inoculum was prepared by mixing about 50mg of *Lycopodium* spores with 2,5mg uredospores of the fungi. A total density of about 150 uredospores per cm^2 is achieved this way. Preferably, the uredospores were collected as closely to the inoculation moment as possible. However, several host plants were contaminated with powdery mildew. To prevent powdery mildew infection during the experiments, the collected rusts spores were

transferred to a desiccator for 3 days. Powdery mildew spores are less viable after storage in the desiccator than fungi spores (Niks, RE., personal communication). In some instances, multiplication on the host plants was not sufficient to acquire 2.5mg of spores. When this was the case, spores were added from the liquid N_2 storage (-196°C). Prior to inoculation, these spores were subjected to flash thowing by dipping them in warm water to prevent damage by ice crystals.

Inoculation

Inoculations were performed in a settling tower (Figure 6). Per round, one box was inoculated with one rust species. A box was placed on the rotating base of the tower and put into the settling tower. The inoculum was then sprayed across the box in a uniform density. The spores were allowed to settle 5-10min. Afterwards, the boxes were placed in a dark humidity chamber (100% relative humidity) to allow the spores to germinate. After each round of inoculation with a pathogen species, the settling tower was cleaned and allowed to dry for 30min before an inoculation with a different rust species was started. Multiple inoculations were done during a day.

To prevent cross infection during the phenotyping, all tools were disinfected with alcohol (70%) and dried with compressed air.

Figure 6. Picture of a settling tower. The settling tower allows the spores to be sprayed over the plants in a uniform density. Boxes are inserted at the base of the tower.

Figure 7. Microscope view of rusts spores and their germination tube. The larger spores are *Lycopodium* spores.

The next day, the boxes were collected and the iron staples were removed. The amount of germinated spores was estimated under the light microscope, by counting the amount of spores with germination tube on the glass slides (Figure 7). This provided a measure by which the success of the inoculation could be estimated. The boxes were transferred to greenhouse compartments with a 16:8 light:dark period and a ambient temperature of about 15 - 18°C. The differences in resistance are best expressed at relatively low temperatures during the development of the colonies.

After about 10 days, when the sporulating pustules were clearly visible, the plants were evaluated for resistance by counting the number of sporulating pustules on the primary leaves and measuring the width and length of those leaves in centimeter. Counting was done with a 10x magnification pocket lens. The infection frequency (IF) and relative infection frequency (RIF) in relation to the susceptible control (SusPtrit) was then calculated.

The IF per cm^2 was calculated by (Equation 1).

$$\text{Eq. 1: } IF = \frac{\#\ pustules}{width\ x\ length}$$

width: width of leaf in cm, length: length of leaf in cm

From the IF, the RIF was calculated (Equation 2).

$$\text{Eq. 2: } RIF = \frac{IF_{genotype}}{IF_{SusPtrit}}\ x\ 100$$

$IF_{genotype=}$= IF of a specific genotype, $IF_{SusPtrit}$= IF of SusPtrit

Data were recorded with Microsoft Excel (2010). During this study, two complete replications were performed. One replication consists of a total of six boxes, each inoculated with a different rusts species. Phenotyping data from this study were added to the first replication, done by Salunke (2013), resulting in three complete replicates. In addition, a 4^{th} replication was done for several homorecombinants, because the IF/RIF of these recombinants varied extremely between the first three replicates. The IF of eight plants per genotype, instead of two, was measured in that replication.

2.1.2 Marker development, primer design and genotyping

In previous studies, SusPtrit, SusQ11 and Steptoe were genotyped with the 9K i-select Infinium array (around 7.500 SNP loci) (Yeo et al, unpublished). This SNP array is based on barley Expressed Sequence Tags (ESTs) sequences. 2639 polymorphic loci between SusPtrit and Steptoe, and 186 between SusPtrit and SusQ11 were found. Those 186 loci were mapped to chromosome 2H of the barley genome, between 88.7 and 146.5cM according to data of the consensus map of the research group. Therefore, the introgression of Steptoe containing *Rphq11* was estimated to be 57.8cM and containing 186 SNP loci from the array. The previous study done by Yeo et al. (unpublished data) on the fine mapping of *Rphq11,* based on the resistance to *Ph*, covered the area between 88 and 119cM using molecular markers not included in the SNP array. Later, when the array was available, fine mapping of the resistance to heterologous rusts covered the region between 105 and 146.5cM (Salunke, 2013). 20 SNP loci were selected in intervals of 5cM in that area (105-146.5cM). Peak marker 25 was, via a substitution mapping approach, found to be associated with *Phs*/*Phb* resistance. The next upstream marker was located at 128cM, and was not found to be associated with the resistance (Salunke, 2013).

In the present study new SNP markers were developed between 121.05cM and 128.13cM, as this region showed to be correlated to *Phs/Phb* resistance. SNP markers were genotyped using Cleaved Amplified Polymorphic sequences (CAPs) and LightScanner.

A CAPs assay uses amplified DNA fragments that are digested with a restriction enzyme. The restriction enzyme recognition site contains the SNP. Therefore, DNA fragments containing a certain allele are digested whereas DNA fragments containing the other allele are not digested. On an agarose gel, these differences are made visible by size separation of the bands (information taken from the NCBI website, consulted in February 2014).
LightScanner is a program which enables the analysis of DNA melting curves. It is a high resolution melting analysis which performs high-throughput gene scanning and mutation detection. It is based on melting differences in the PCR products which are caused by the SNP. It is an easy system which does not require post-PCR reagent addition or separation (Yuan et al., 2009).

In addition, KASPar markers were designed for future recombinant screenings. The KASPar genotyping is a homogenous fluorescence (FRET) based assay that is a unique form of allele specific PCR (information taken from the LGC genomics website, consulted in February 2014). It provides a way to quickly screen a large number of DNA samples. It is useful in screening a large recombinants population on the basis of specific alleles.

The development of the SNP markers & primers and genotyping was done in six steps (Figure 8), based on Salunke (2013):

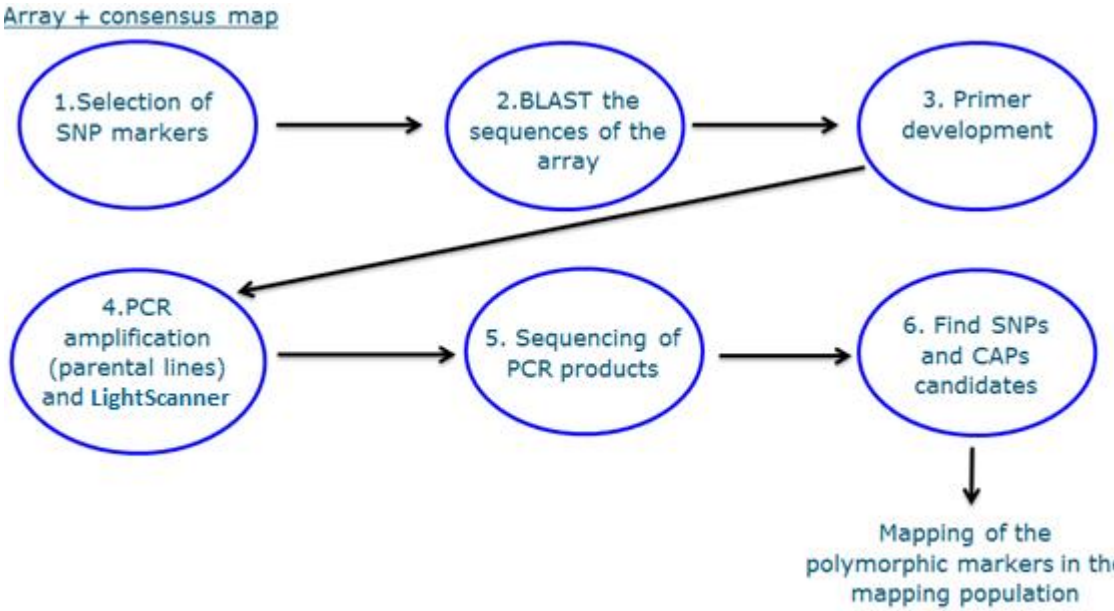


Figure 8. Schematic overview of the steps taken in the construction and the mapping of the SNP markers.

1. SNP loci were selected from the array based on their location on the consensus map and their polymorphism between SusPtrit and SusQ11. Also, for those selected markers, there was no polymorphism between Steptoe and SusQ11.

2. The array sequences containing the SNPs (between 180 and 250bp) were blasted (megablast, nr/nt) in the NCBI database to homologous sequences in barley (or, if not available, in wheat or rice). In this way, a bigger sequence was available on which primers could be constructed. The location of the SNP flanking regions in the sequence was then confirmed in the found sequence, using the array sequence.

3. Primers were then designed with Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/) using the blast sequence. The SNP was always in the middle of the amplicon to (i) allow visual size differentiation in an agarose gel following CAPs, (ii) allow reliable sequencing of the SNP and its flanking regions and (iii) to allow polymorphism detection in the melting curves with LightScanner. The primers and amplicon had the following characteristics:

- Melting temperature (Tm) primers: 58-62ºC (optimum 60ºC)
- Primer size: 18-30bp (optimum 20bp)
- G+C content: 40-60% (optimum 50%)
- Amplicon size: 100-500bp
- Tm amplicon: 70-90ºC (optimum 75ºC)
- Poly-N max = 3
- No T in 3' end

Moreover, the quality of the potential primers was checked using the Netprimer (http://www.premierbiosoft.com/netprimer/index.html) software. The primers were restricted to the following minimums:

Hairpins:
3`end: -2kcal/mol
Internal: -6kcal/mol

Self-dimers:
3`end: -5kcal/mol
Internal: -6kcal/mol

Cross-dimers:
3`end: -5kcal/mol
Internal: -6kcal/mol

One primer pair per SNP was designed.

The sequences found with the blast, used for primer design, were checked for CAPs candidates using the dCAPs Finder 2.0 programme (http://helix.wustl.edu/dcaps/).

4. A Polymerase Chain Reaction (PCR) was performed using the primers on parental DNA (in this case Steptoe, SusPtrit, SusQ11 and a simulated heterozygous sample (Steptoe + SusPtrit DNA mixed in a 1:1 ratio)). This DNA was readily available from previous research (Salunke, 2013). A PCR protocol optimized for the LightScanner methodology was used (Table 3; Figure 9). PCRs were performed in 96-wells plates, using Bio-Rad PCR machines.

Table 3. The composition of the master mix used for the PCR reaction for the LightScanner. Volumes are given for one sample.

Component	Volume (µl)
MQ (demineralized water)	10
5x reaction buffer for the Phire enzyme	4
LC-green	2
dNtps	0.8
Forward primer (5µM)	0.5
Reverse primer (5µM)	0.5
5x Phire enzyme	0.2

DNA (7.5 ng/µl)	2.2
Mineral oil	20

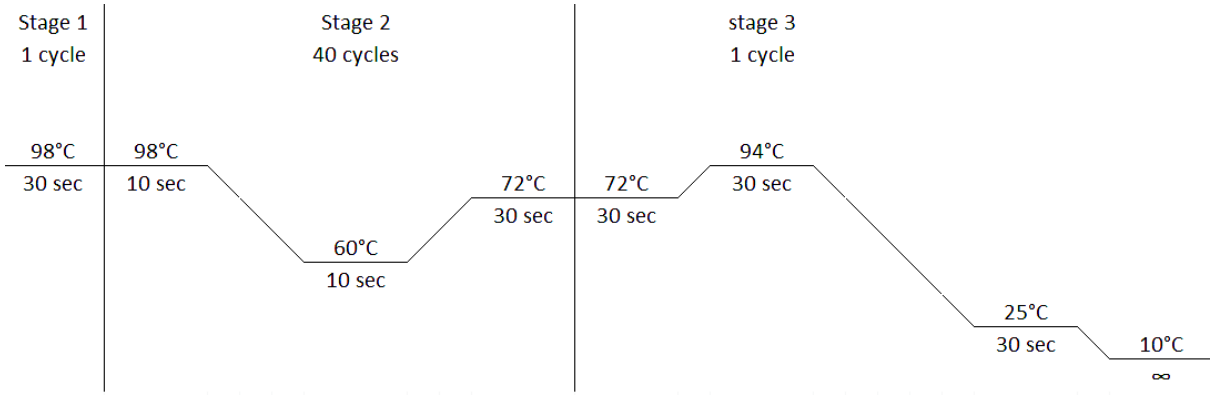


Figure 9. Schematic representation of the PCR LightScanner program with the amount of cycles and melting temperatures (Tm).

After the PCR, the samples were loaded into the LightScanner to determine the melting curves and to determine whether any PCR products were present, and in this case whether there were polymorphisms between the parental lines. The presence of an amplicon was observed by the presence of fluorescence (higher than 800 on the scale of LightScanner).

5. PCR products were then run on an agarose gel (1.5%; 0.5x TBE Buffer; 90volts; ±1h) to check the size, number and quality of the amplicons. GelRed was used to stain the DNA (concentration DNA:Gelred 5:1) throughout this study. The GeneRuler™ 1kb plus DNA ladder was used in sizing the DNA samples in this study. Samples were then sequenced to identify whether the primers amplified the correct sequence. Samples were then either (i) send directly for sequencing if a single clear band was observed (ii) purified and send for sequencing if a single weak band was observed or (iii) if multiple bands were observed, the band of the appropriate size (on the basis of the primer design) was cut from the agarose gel, purified and send for sequencing. If cutting was not possible due to the presence of too many bands, the marker was discarded from the pileline. PCR purification was performed using the QIAquick® PCR Purification kit. The MinElute® Gel Extraction Kit was used to purify the DNA which was cut from the agarose gel. The manufactures protocol was used when using the kits. Sequencing was performed by GATC Biotech, using their provided protocol. In this study, the DNA concentration was measured using NANOdrop.

6. Sequences were studied with the Chromas programme (http://technelysium.com.au/ - version 2.4.1) to check the quality of the sequencing and the homozygous/heterozygous state of the potential SNPs in the different genotypes. Sequences were aligned and screened for SNPs using the MEGA software (http://www.megasoftware.net/ - version 5.10). Sequences were screened for the presence of the array SNP and/or new SNPs. Primers could be used if at least one polymorphic SNP was present in the amplicon. Sequences were blasted again to identify whether the blast result was identical with the blast result from step 2. CAPs candidates were confirmed using dCAPs finder 2.0.

The PCR products were analyzed with LightScanner. If polymorphisms of the parental lines were detected with the LightScanner, the mapping population (in this case the homorecombinants) was genotyped using only LightScanner. In this study. products were deemed polymorphic when melting

curves could be assigned to three groups based on the parental lines. In this case these parental lines where Steptoe, SusPtrit and the additional heterozygous sample containing both parents. If the parental lines were polymorphic with LightScanner, it was theoretically possible to genotype the mapping population also with LightScanner. Controls of the parental lines were always added to the wells plate during mapping population screening. However, polymorphisms detected in the parental lines were not always detectable in the mapping population. If the LightScanner results were monomorphic in the parental lines of mapping population, or to corroborate the LightScanner results, the PCR product was digested using the CAPs technique. The components for the master mix for CAPs can be found in table 4. Restriction was done overnight (37°C). After that, the samples were electrophoresed for 1,5h in an agarose gel at 2.5% (0.5x TBE, 90volts). If the parents were polymorphic, the mapping population was genotyped using CAPs. Bands were scored for the presence of a specific allele visually.

Table 4. The composition of the master mix used for the CAPs technique.
Volumes are given for one sample.

Components	Volume (µl)
PCR product	10
MQ	16
10x buffer*	3
Enzyme	1

* buffers are enzyme specific

KASPar markers were developed using sequences obtained in this study and sequences from Salunke (2013). SNPs for KASPar genotyping need to have 50 monomorphic bases upstream and downstream of the SNP. Markers flanking *Rphq11* and the *Phs/Phb* QTL were selected, as closely to the QTLs as possible.

2.1.4 Histological assessment

The histological study was done to study the response of barley to an infection by *Phb_Iran* in detail and to see whether the histological effect of the defense response of barley varied between barley genotypes containing different combinations of the QTLs *Rphq11* and *Phs/Phb*. To this end, barley was inoculated with *Phb_Iran* and subsequently stained to make the infections visible with an UV microscope.

It was tried to multiply the rust on *Hordeum bulbosum*, however the rusts failed to produce the quantity of uredospores needed for the inoculation (5mg). As a result, spores were taken from the liquid N_2, as described in §2.1.1.

One box was prepared for inoculation(§2.1.1). Five barley genotypes were used, which were selected on the basis of their alleles in the QTLs (Table 5). 10 seeds per genotype were planted. Four plants to study the histological response and six to measure the IF. The inoculum was prepared by mixing 5mg of uredospores with about 50mg *Lycopodium* spores. Seven days after inoculation a central segment of about 3cm of the primary infected leaf was taken from the four plants per genotype. These leaf segments were directly transferred to tubes containing a lactophenol-ethanol solution (1:2 v/v), for staining. Three days after leaf collection, the remaining leaves were evaluated.

The amount of pustules was counted and the width and length of those leaves in cm was measured. The IF and RIF was calculated as described in §2.1.1.

Table 5. Barley genotypes selected for the histological study. Genotypes were selected on the basis of their alleles for the QTL *Phs/Phb* and *Rphq11*. An "A" allele indicates the Steptoe allele, a "B" allele indicates a SusPtrit allele.

Genotype	Allele *Rphq11*	Allele *Phs/Phb* QTL
Steptoe	A	A
SusPtrit	B	B
SusQ11	A	A
Homorecombinant 7	A	B
Homorecombinant 19	B	A

Staining

The lactophenol-ethanol solution consisted of 250ml lactophenol (250ml lactic acid, 500ml glycerine and 250ml 20% phenolsolution) and 500ml ethanol (96%). The phenol solution (20%) was prepared by mixing 50ml phenol solution (99%) with 200ml MQ. The leaf material was immediately after collection fixed and bleached by boiling it for 1.5min in a water bath in the lactophenol-ethanol. Samples were then left overnight.

The following day, leaf segments were (i) washed for 30min in ethanol (50%) by replacing the lactophenol-ethanol, (ii) washed for 30min in 0.05M NaOH (2g/l), (iii) rinsed 3x with MQ and (iv) washed for 30min in 0.1M Tris/HCl buffer (pH 8.5). The buffer was prepared by dissolving 12.1g Tris in 800ml MQ. pH was adjusted to 8.5 by adding HCl (75%). MQ was then added until a final volume of 1L was reached. Staining was done for 5min with Uvitex (0.05% in Tris/HCl buffer). Afterwards, the segments were rinsed 4x with MQ and washed for 30min in a solution of 25% glycerol. Samples were prepared for microscopy by putting the leaves on object slides, longitudinal to the axis of the leaf, embedded in glycerol. Two leaves were put on one slide.

Under the UV microscope (40x magnification; 1x zoom), the number of early aborted (EA) infections with and without necrosis and the number of established infections (EST) with and without plant cell necrosis (recognized by yellow fluorescence) was counted on the leaf segments. Established infections were defined as having at least six haustorial mother cells. Early aborted infections were defined as having fewer than six haustorial mother cells. Method adapted from Niks (1982), Niks (1983a) and Niks (1983b). A total of 50 units were recorded. The amount of established infections containing sporogenic tissue (red fluorescence) was also counted. The width of the established infections was measured in µm (10x magnification; 1x zoom). The width of overlapping infections was not measured. It should be noted that larger infections show more natural overlap than smaller infections. The width of the infections is therefore somewhat undervalued. Pictures were made using a CANON Powershot A620 and the Axiocam software. Pictures were stacked with the Zerene stacker software. A Zeiss UV microscope was used.

2.1.5 Data analysis

Data were recorded in Microsoft Excel (2010). Data were analyzed using Genstat 16th edition and SPSS 20th edition. In Genstat, model assumptions were checked using residual plots (equal variances, normality). In SPSS, the Shapiro-Wilk test was used to asses normality and the Levene's test to test equality of variances. The assumption of independent observations was not violated, due to the experimental set-up.

A QTL analysis was performed with the phenotypic data of all four replicates. 17 markers, evenly spread across the Steptoe introgression in SusQ11 (Salunke, 2013), were individually analyzed for all four replications with an two-way Analysis Of Variance (ANOVA). This is also called a "single-marker analysis". The following model was constructed (Equation 3) with two factors (allele and replicate):

$$\text{Eq. 3: } y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \underline{e}_{ijk}$$

i = 1,2; j = 1,2,3; k=1..23

α = effect of the allele i, β = effect of replicate j, αβ = interaction of the i^{th} allele and the j^{th} replicate,

y_{ijk} = relative infection frequency (RIF)

$$e_{ijk}\ i.i.d. \sim N(0, \sigma_e^2)$$

This test showed whether the average RIF differed between the alleles, between the replicates and whether an interaction existed. In the end, P-values were converted with the following formula: -^{10}log(*probability*). The critical p-value (α) was taken at 0.001 in case of the main effects. The interaction was deemed significant at the 0.05 level, as these can generally influence results greatly. It was expected that in case of a significant association of a marker with RIF, the Steptoe allele would be associated with low RIF and the SusPtrit allele with high RIF.

Next to the QTL analysis, a substitution mapping procedure was performed on the phenotypic data by comparing the RIF of the different genotypes (homorecombinants + controls) with SusPtrit and SusQ11. Comparisons were made between individual plant measurements and SusPtrit mean RIF. An two-way ANOVA (Equation 3) was run to test the effect of genotype, individual plant and replicate on the RIF. The only difference with equation 3 being α, which indicates here "genotype", having 23 levels. RIF was ^{10}log+1 transformed to achieve normality of the residuals and to correct for minus logarithms. A Dunnetts test was used as multiple comparisons test, to test whether the average RIF per genotype was significantly different from SusPtrit and/or SusQ11. The critical p-value was 0.05. A subNIL with an RIF value statistically significantly lower than the RIF of SusPtrit and identical to SusQ11 were considered to carry the SusQ11 allele, whereas a subNIL with an RIF value not statistically different from the RIF on SusPtrit and different from SusQ11 were considered to carry the SusPtrit allele. Some intermediate cases were identified, which led to alleles that could not be identified. This method was adapted from Marcel et al. (2007a). The found allele "patterns" were associated visually with a marker map available for the genotypes.

To investigate whether a two-locus interaction was present between *Rphq11* and the 121-128cM region (or possible other regions associated with heterologous rusts resistance), RIF values were averaged for each allele combination of *Rphq11* and the other QTL(s). If four or three distinct levels were distinguished, with increasing RIF if more SusPtrit alleles were present, an interaction was declared to be present.

For the histological data, the measured width of EST infections was ^{10}log transformed to achieve statistical normality. Data were analyzed using a one-way ANOVA (Equation 4) with one factor (genotype). A Fisher's protected LSD was run as post-hoc test. The critical p-value was 0.05.

$$\text{Eq. 4: } y_{ij} = \mu + \alpha_i + \underline{e}_{ij}$$

i = 1….5, j = 1…x (x depends on the amount of observed established infections)

α = effect of genotype i, y_{ijk} = width of established infection

$$e_{ijk}\ i.i.d. \sim N(0, \sigma_e^2)$$

The counted number of established infections was transformed to a fraction of total infections (%EA; Equation 5). %EST is complementary to %EA and is therefore not calculated.

$$Eq.\,5: \%EA = \frac{\#EA}{\#EA + \#EST}$$

Data were Arcsin transformed to allow the residuals to follow a normal distribution. Data were analyzed using a one-way ANOVA, as can be seen in equation 3. The only difference was the y-variable (proportion early aborted infections) and j having only 4 levels. A Fisher's protected LSD was run as post-hoc test. The critical p-value was 0.05. The same model was run for the proportion of established infections containing sporogenic tissue.

The RIF of the histological data were ^{10}log transformed to achieve a normal distribution of the residuals. Data were analyzed using a one-way ANOVA, as can be seen in equation 3. The only difference was the y-variable (IF) and j having 6 levels. A Fisher's protected LSD was run as post-hoc test. The critical p-value was 0.05.

2.2 Resistance to *Pp* and *Phb* in L94 background

Progeny of the 1501-2 to 1501-5 barley genotypes (coming from the cross L94xSusPtrit) was available (n=170). The 1501-2 to 1501-5 parental plants were shown to be heterozygous for the markers 3, 7, 8, 13, 25, 26 and 36 (Table 6). Markers 3, 8 and 26 were shown to be associated with *Pp* resistance and marker 8 and 13 were shown to be associated with resistance to *Phb* (Chisenga, 2013). The 1501-2 to 1501-5 plants were selfed to allow recombination between these markers of interest. Seeds were harvested and sown for phenotyping, genotyping and for getting offspring for future studies.

Table 6. Overview of the barley genotypes 1501-2 to 1501-5 with the marker associated with resistance to *Pp* and *Phb_Isr*. Some unused markers are indicated for comparison with Chisenga (2013). Abbreviations: M=marker, H=heterozygous (L94 and SusPtrit alleles). Data is based on sequencing and LightScanner genotyping.

	M: 3	M: 7	M: 8	M: 25	M: 26	M: 36	M: 13
Genotype	2H:123.34	2H:132.3	2H:132.3	6H:60.63	6H:60.63	6H:60.63	5H:10.62
1501-2	H	H	H	H	H	H	H
1501-3	H	H	H	H	H	H	H
1501-4	H	H	H	H	H	H	H
1501-5	H	H	H	H	H	H	H

2.2.1 Phenotyping

Phenotyping was performed on the progeny of 1501-2 to 1501-5. Individuals were phenotyped with *Pp* and *Phb_Isr*; the same protocol as described for *Rphq11* was used. A total of 170 individuals were phenotyped. As controls, 4-5 seeds of Vada, L94 and SusPtrit were sown per box. About 40 plants, including controls, were sown per box.

2.2.2 DNA isolation

DNA was extracted from frozen leaf material (-80°C) of the 1501-2 to 1501-5 progenies. DNA was extracted according to the RETCH 1.3 protocol of the department of Plant Breeding. The day before the extraction the buffers were prepared. Lysis buffer was prepared by mixing 100ml Tris/HCl 1M (pH 7.5), 5ml EDTA 0.5M (pH 8), 200ml NaCl 5M and 195ml MQ and dissolving 10g CTAB. Extraction buffer was prepared by mixing 50ml Tris/HCl 1M (pH 7.5), 5ml EDTA 0.5M (pH 8) and 445ml MQ and dissolving 31.9g Sorbitol. 5% Sarkosyl was prepared by dissolving 5g Sarkosyl in 100ml MQ. Isolation buffer was prepared by mixing 84ml lysis buffer, 84ml extraction buffer, 33.5ml Sarksoyl 5% and 20µl RNase (100 mg/ml) and dissolving 500mg Sodium bisulfide (NaHS). On the day of the extraction, the isolation buffer was heated for 30min in a 60°C water bath.

The full grown progeny of the 1501-5 plant was available. Leaf material was collected for DNA isolation. The youngest leaves were cut, carefully transferred to microcentrifuge tubes and put in liquid N_2. The material was then stored at -80°C. Tools were cleaned with alcohol (70%). Leaf material of the other progenies was collected prior to this study and stored at -80°C.

From the stored leaf material, about the size of two leaf blades was cut. Samples were put on liquid N_2. Tubes containing the leaf material were filled with glass beads. Samples were milled for 30sec at 30Hz three times. Afterwards, the samples were centrifuged and isolation buffer (400µl per tube) was added. Samples were then incubated in a water bath of 65°C for 60min. Samples were mixed every 10min. After incubation, 400µl of chloroform:isoamylalcohol (24:1) was added and samples were mixed for 5min. Phases were separated by centrifuging at 4600rpm for 30min. Two times 150µl was taken from the liquid phase of the samples and put into new tubes containing 300µl isopropanol. The DNA was pelleted by centrifuging for 30min at 4600rpm. Afterwards, the supernatant was discarded and 200µl of 75% ethanol added. Again, the samples were centrifuged (20min, 4600rpm). The supernatant was discarded and the pellets were dried for around 1h. The next day, the pellets were dissolved in 100µl MQ, 1µl of RNase was added and samples were left at room temperature for 30min. The quality of the DNA was checked on an agarose gel (1%, 90 volts, about 1h).

In the end, 173 DNA samples were extracted. DNA of some plants was extracted in duplicate as leaf quality of these plants was low.

2.2.3 Genotyping

LightScanner and CAPs were used for genotyping, as described in the *Rphq11* section. Parental DNA was always used as controls in genotyping the mapping population. Markers 3, 7, 8, 13, 25, 26 and 36 were run; using the primers developed by Chisenga (2013) (Table 45). In the previous study, marker 36 was found to be associated with resistance in L94; average phenotypic values were identical for the individuals that were homozygous for the L94 alleles and individuals that were heterozygous (Chisenga, 2013). This suggests dominance of the L94 allele. This marker was run again to corroborate those results.

2.2.4 Data analysis

A chi-square test for goodness of fit was carried out for each individual marker to check if the alleles were segregating according to the expected Mendelian ratios (1:2:1 in the case of co-dominant markers).

A one-way ANOVA was carried out to check the marker association between phenotype (RIF) and the allele of the markers (either L94, heterozygous or SusPtrit allele) (Equation 6). Each marker was individually analyzed.

$$\text{Eq. 6: } y_{ij} = \mu + \alpha_i + \underline{e}_{ij}$$

i = 1,2,3; j = 1..170

α = effect of the allele i, y_{ijk} = RIF

$$e_{ijk}\ i.i.d. \sim N(0, \sigma_e^2)$$

A Fisher's protected LSD was run as post-hoc test. A critical value of 0.05 was taken. It was expected that in case of a significant association of a marker with RIF, the L94 allele would be associated with low RIF and the SusPtrit allele with high RIF. The heterozygous allele was surmised to be intermediate.

3. Results

3.1 SusQ11

3.1.1 Genotyping

For the fine mapping of the 121-128cM region, a total of 14 primer pairs were each designed based on 14 SNPs derived from the array (Table 7 & Appendix §7.2.1). Of these markers, a total of 12 markers produced clear amplicon(s) for the parental lines on a gel (Figure 10). Two samples (63 and 70) were discarded from the pipeline, as the marker primers produced no amplicon (observed with LightScanner). Marker 65 had too many amplicons, and was therefore not used in the study.

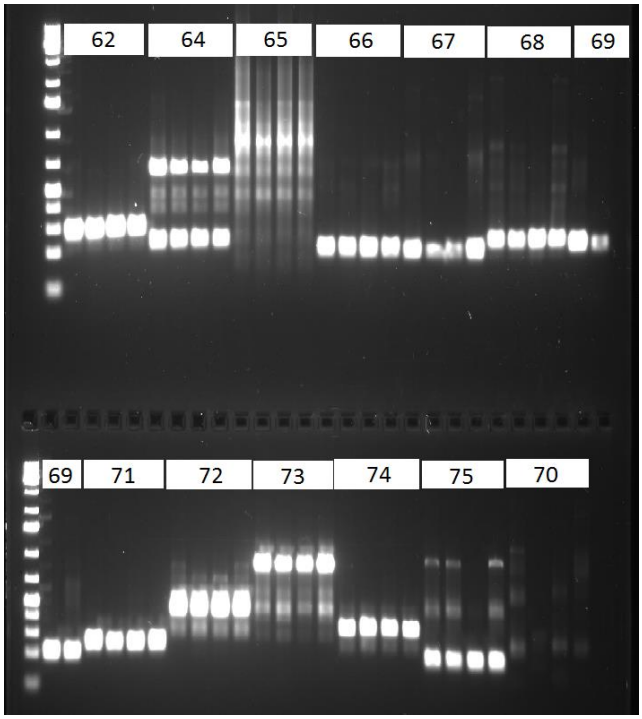


Figure 10. Gel showing the PCR products of 13 SNP markers of the parental lines Steptoe, SusPtrit, SusQ11 and the heterozygous samples Steptoe + SusPtrit. For each markers, samples are ordered as mentioned above. The first well of each row is filled with the GeneRuler™ 1kb plus DNA ladder.

Sequences were obtained for 11 markers; an example of an alignment is given in figure 11. For most of the markers, the correct SNP was amplified; as indicated by an clear alignment of the array sequence and the amplified sequence and the presence of the same SNP bases. The amplicon of marker 67 was shifted. The SNP was located close to the start of the amplicon. A different sequence was therefore amplified which contained no useful polymorphic SNPs. The sequence of marker 73 could be aligned to the array sequence that was the basis for marker 74. This event also occurred the other way around, for marker 74. These samples are therefore likely switched (Table 7). This had no direct effect on the genotyping results.

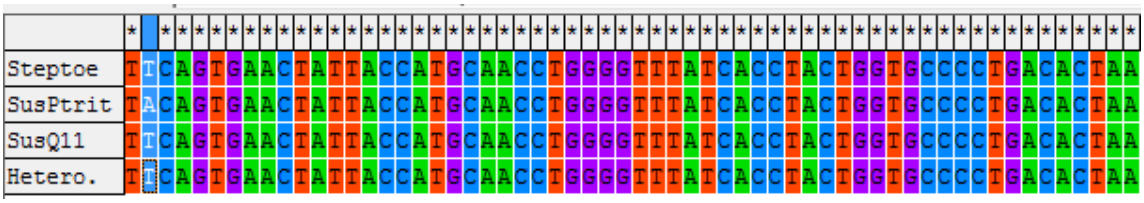


Figure 11. Example of aligned sequences for the 4 parental samples, for marker 62 (view of MEGA prgramme). Only a part of the sequences are displayed. The SNP is located in the beginning of the sequences (highlighted blue). This is an example of an polymorphic marker, with a different allele for the parents Steptoe and SusPtrit and an identical allele for Steptoe and SusQ11. The heterozygous sample contained both alleles (observed with Chromas); but MEGA displayed only one.

Samples that were polymorphic with LightScanner (a total of 9 markers) were mapped in the mapping population of the homorecombinants. In the end, 3 markers were reliably scored with LightScanner (Table 7). Results are given in table 8.

A total of 7 markers were polymorphic for genotyping by CAPs in the parental lines (Table 7). These markers were mapped in the homorecombinants. An example of the CAP genotyping is given in figure 12. The results are given in table 8.

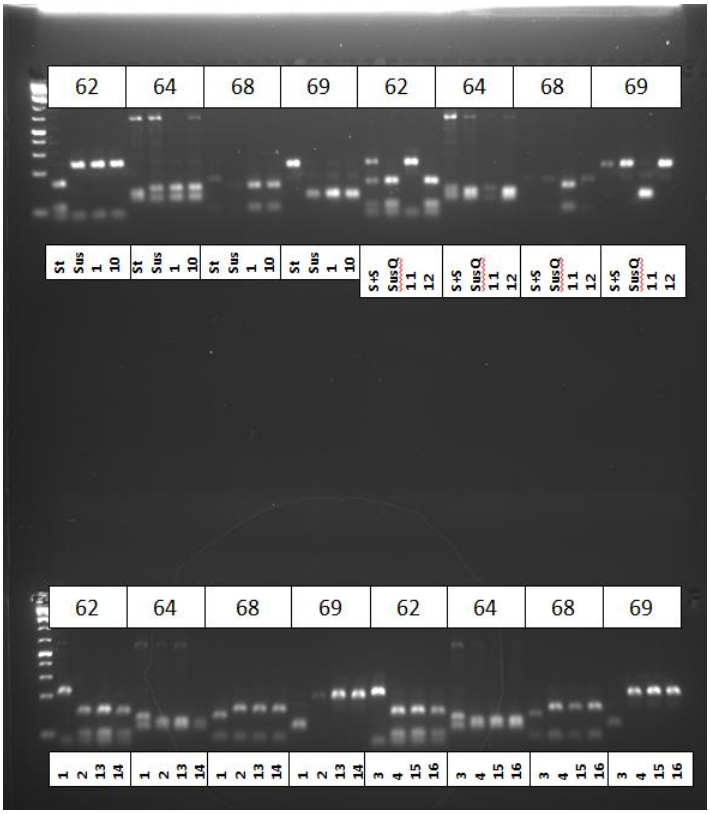


Figure 12. Example of a CAPs result for marker 62, 64, 68 and 69. Samples are indicated below the bands. The first well of each row is filled with the GeneRuler™ 1kb plus DNA ladder. Steptoe. SusPtrit, SusQ11 and the heterozygous samples are denoted with "St", "Sus", "SusQ" and "S+S" respectively.

Table 7. List of the used SNP markers with their respective: (i) locus name on the 9K i-select Infinium array, (ii) blast result of the array sequence, (iii) blast results of PCR amplicon sequence, (iv) location on the consensus map (CM), (v) Oregon Wolf Barley map (OWB) and (vi) on a other consensus map (provided by i-select), (vii) amount of PCR products, (viii) number of SNPs in the sequence of the PCR product, (ix) indication whether the markers were polymorphic (PM) in the parental lines (PL) with LightScanner, (x) indication if the markers were genotyped in the mapping population (MP) with LightScanner (Yes=genotyped), (xi) CAPs candidate of the PCR sequence, (xii) indication whether the marker was polymorphic in the PL with CAPs, (xiii) indication if the markers were genotyped in the MP with CAPs. Blank cells indicate either absence of product, no polymorphism or no CAPs candidate. One asterisk (*) indicates switched samples, occurred during sample handling; two asterisks (**) indicate that the results from the blast of the array sequence and the amplified sequence were not similar. However, in this case the sequences of the two results were almost identical. NA = enzyme not available at the department.

Marker				Map Position			(vii) PCR		(ix) Lightsc.	(x) Lightsc.	(xi) CAPs	(xii) CAPs	(xiii) CAPs
name	(i) Locus name	(ii) Blast (array)	(iii) Blast (seq)	(iv) CM	(v) OWB	(vi) Other	amplification	(viii) # SNPs	PL	MP	candidate	PL	MP
62	**SCRI_RS_48964**	AK370277.1	AK370277.1	121.05		106.44	Single band	1			HinfI, Tsp4CI	PM	Yes
63	**SCRI_RS_116575**	AK354414.1		121.05		113.48							
64	**BOPA1_6996-838**	AK356168.1	AK356168.1	121.05			Two bands	2	PM		HpyCH4V	PM	Yes
65	**SCRI_RS_223885**	AK362512.1		121.25		106.44	Some bands						
66	**BOPA1_111-499**	AK251678.1	AK251678.1	121.48	132.56	112.91	Single band	1	PM		Hpy188I	NA	
67	**BOPA2_12_31402**	AK250572.1	AK250458.1**	123.16	132.56	113.48	Single band	1	PM				
68	**BOPA1_871-462**	EF514912.1	EF514912.1	123.34	132.56	113.48	Single band	1	PM		BsuRI	PM	Yes
69	**SCRI_RS_139831**	AK357373.1	AK357373.1	123.72		107.15	Single band	2	PM	Yes	Hin1II	PM	Yes
70	**SCRI_RS_224624**	XM_003561284.1		123.72		111.94							
71	**BOPA2_12_21396**	AK374410.1	AK374410.1	124.51		113.92	Single band	1	PM				
72	**SCRI_RS_222093**	AK365472.1	AK365472.1	126.62		107.15	Single band	1	PM				
73	**BOPA1_14832-296**	AK373283.1	AK362157.1*	126.62		108.22	Two bands	0	PM	Yes			
74	**BOPA1_3180-1771**	AK362157.1	AK373283.1*	126.62	133.80	115.08	Single band	1	PM	Yes	AluI	PM	Yes
75	**SCRI_RS_219799**	AK369787.1	AK369787.1	127.53		108.00	Three bands	1			XhoI	PM	Yes

In the end, 7 markers were mapped on the 121-128cM region of chromosome 2H (Table 8; Figure 13). The markers are 62, 64, 68, 69 73, 74 and 75. All markers mapped the same alleles (SusPtrit or Steptoe) in the recombinants; for example all markers indicated a SusPtrit allele for homorecombinant 7. Mapped alleles were identical to marker 25 (Figure 17) as mapped by Salunke (2013), hence no recombinations were found with the seven markers.

Table 8. Genotyping results of 7 SNP markers for the region 121.05cM to 127.53cM of chromosome 2H of Barley. Genotyping was performed by either CAPs or LightScanner (LS). Rec 13' was not genotyped. An "A" indicates the Steptoe allele, a "B" indicates the SusPtrit allele, Rec = homorecombinant. For each marker, the locus name of the array is given.

Position (cM)	121.05	121.05	123.34	123.72		126.62	126.62		127.53
Locus	SCRI_RS_48964	BOPA1_6996-838	BOPA1_871-462	SCRI_RS_139831		BOPA1_14832-296	BOPA1_3180-1771		SCRI_RS_219799
Marker	62	64	68	69		73	74		75
Genotype/Type	CAPs	CAPs	CAPs	LS	CAPs	LS	LS	CAPs	CAPs
Steptoe	A	A	A	A	A	A	A	A	A
SusPtrit	B	B	B	B	B	B	B	B	B
SusQ11	A	A	A	A	A	A	A	A	A
Rec. 1	B	B	B	B	B	B	B	B	B
Rec. 2	A	A	A	A	A	A	A	A	A
Rec. 3	B	B	B	B	B	B	B	B	B
Rec. 4	A	A	A	A	A	A	A	A	A
Rec. 5	B	B	B	B	B	B	B	B	B
Rec. 6	B	B	B	B	B	B	B	B	B
Rec. 7	B	B	B	B	B	B	B	B	B
Rec. 8	B	B	B	B	B	B	B	B	B
Rec. 9	B	B	B	B	B	B	B	B	B
Rec. 10	B	B	B	B	B	B	B	B	B
Rec. 11	B	B	B	B	B	B	B	B	B
Rec. 12	A	A	A	A	A	A	A	A	A
Rec. 13	A	A	A	A	A	A	A	A	A
Rec. 14	A	A	A	A	A	A	A	A	A
Rec. 15	A	A	A	A	A	A	A	A	A
Rec. 16	A	A	A	A	A	A	A	A	A
Rec. 17	A	A	A	A	A	A	A	A	A
Rec. 18	A	A	A	A	A	A	A	A	A
Rec. 19	A	A	A	A	A	A	A	A	A
Rec. 20	B	B	B	B	B	B	B	B	B

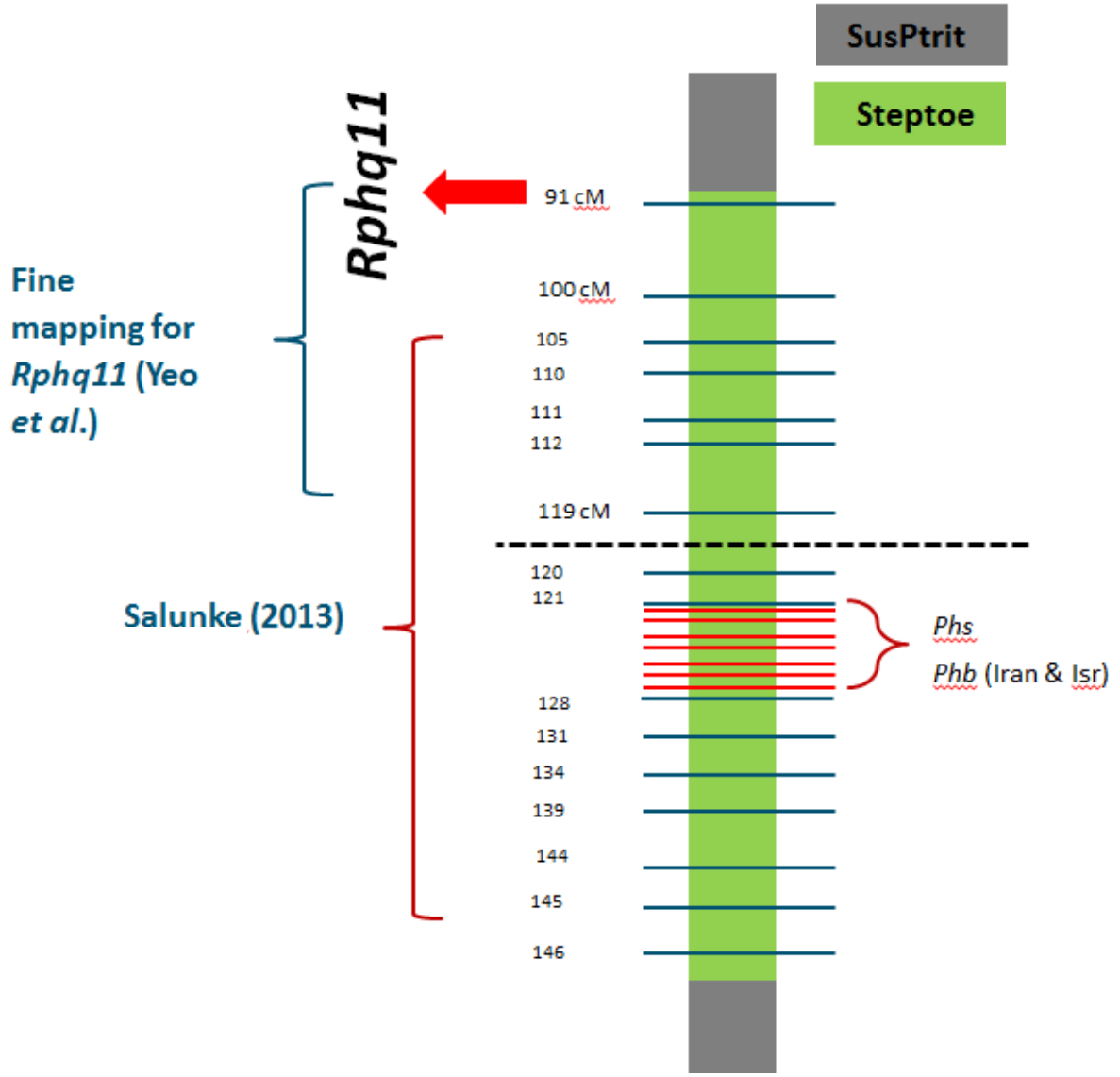


Figure 13. Overview of chromosome 2H of SusQ11. The Steptoe introgression is indicated in green, the SusPtrit background is indicated in grey. Blue lines indicate examples of previously constructed markers by either Salunke (2013) or Yeo et al. (unpublished) and red lines indicate the markers constructed in this study. The peak marker for *Ph* resistance (*Rphq11*) is indicated with the red arrow. The peak marker and the subsequent area found to be associated with *Phs*/*Phb* resistance in the study of Salunke (2013) is indicated with the red brace.

KASPar markers

Four KASPar markers were constructed during this study (Table 9). Markers were constructed by selecting markers located upstream and downstream of *Rphq11* and the *Phs*/*Phb* QTL. DNA sequences were then investigated for the presence of polymorphic SNPs which displayed KASPar characteristics. For KASPar genotyping, polymorphic SNPs are needed that are flanked by a region of 50bp upstream and downstream of the SNP which are monomorphic between the parents.

Markers WBE129 and K04002 were selected as flanking *Rphq11* upstream and downstream (Figure 17). Although the exact position was absent on the marker map used in this study, marker locations were confirmed by blasting the sequences on the available barley genome sequence (http://webblast.ipk-gatersleben.de/barley/viroblast.php). The sequence of marker WBE129

contains, next to the SNP, also an insert in the SusPtrit line. However, it was surmised that this would not influence the future KASPar screening (Niks RE., personal communication).

Markers 7 (120cM) and 47 (139cM), developed by Salunke (2013), were selected as flanking markers for the *Phs*/*Phb* QTL (Figure 17). Also, the markers flanked the region indicated as associated with *Pp* resistance (Salunke, 2013). Marker 47 is located somewhat far away from the *Phs*/*Phb* QTL (at least 10cM), however no other markers were available that suited the KASPar characteristics. Marker 7 is located at the same location as marker 8 (Figure 17).

Table 9. Selected markers for KASPar screening on chromosome 2H. For each marker the name, the position, the flanking QTL and the sequence 50bp upstream and downstream of the polymorphic SNP is displayed. The SNP is indicated in red. Alleles are indicated between the parentheses; the Steptoe allele is allele displayed before the slash, the SusPtrit allele is displayed after the slash. For marker WBE129, next to the SNP which is located in the middle of the sequence, a deletion/insertion has been found in the region.

Marker	Position (cM)	Flanking QTL	Sequence
WBE129	89.8	*Rphq11*	CCGTCCACGACTTCACCGTCAAGGTCATCCCCGCTCCCCTCTCCTCCCCTCC(C/T)GTCTCTGTATTTTTTTT(-/T)ATTTCACACGCGCTGCTAGATCCCCTGCTCGG
K04002	94.5	*Rphq11*	TATATGTTCTACCGGACCAAGACGCTAAGCGGCACCGGTGAGAAGCCGGC(A/G)TCAGATGAAGAACATGTTGTCCTGAAGATCCACGTGCAGCGTCGGGAAGT
7	120.65	*Phs*/*Phb*	CAGGAAGACTATGACAGTTTGATGAAGTCTCTACGGGAGAATGATCCTTC(A/G)TGGCCTTCCCTGATGCTGAAGGTGTGATTTTTCCCTTCCTTTTCCTTTAC
47	139.45	*Phs*/*Phb*	TGCCCGAGCGGATCATGAAGCCCTTGTCTGTGAGTTGCCTGGACAGTAAG(G/A)TCGTCGTCGTTGGCCGAGGTCTCCATGTAGTGATCGGCCATATCAAGAAG

3.1.2 Phenotyping

A total of three rounds of phenotyping (three replications) were performed in this study; each at a separate time point. Data were added to the phenotyping results of the first replication (Salunke, 2013). An example of the results is given in table 10. The rest of the data can be found in the appendix (§7.1.2).

Table 10. Example of phenotypic results of the pathogen *Pp* for each homorecombinant and parental line. The measured IF and the RIF is given for each replicate. The average RIF and IF is displayed for completeness. Colors indicate highest and lowest value, from dark green (lowest) to dark red (highest).

Pathogen	Genotype	IF_1	IF_2	IF_3	IF_4	Avg. IF	RIF_1	RIF_2	RIF_3	RIF_4	Avg. RIF
Pp	SusPtrit	9,5	8,5	5,5	5,8	7,3	100	100,0	100,0	100	100,0
Pp	Steptoe	0	0,0	0,0	0,1	0,0	0	0,0	0,5	1,7	0,6
Pp	SusQ11	3,0	0,4	0,3	0,7	1,1	25,0	4,7	6,1	12,1	12,0
Pp	Rec_1_Q11	14,5	4,9	3,3		7,6	185,1	57,4	59,3		100,6
Pp	Rec_2_Q11	2,1	0,7	1,0		1,3	17,9	7,7	18,4		14,7
Pp	Rec_3_Q11	14,1	7,9	2,9		8,3	117,9	93,4	51,9		87,7
Pp	Rec_4_Q11	5,6	1,3	4,3	4	3,8	46,4	15,8	77,4	69,0	52,1
Pp	Rec_5_Q11	7,6	4,9	6,0		6,1	63,0	57,5	108,7		76,4
Pp	Rec_6_Q11	24,8	5,4	4,8		11,7	315,6	64,2	86,7		155,5
Pp	Rec_7_Q11	11,7	6,0	4,3		7,3	97,2	71,3	78,4		82,3
Pp	Rec_8_Q11	7,9	8,8	4,7		7,1	66,2	103,4	85,2		84,9
Pp	Rec_9_Q11	18,8	8,0	4,5		10,4	240,0	94,1	82,3		138,8
Pp	Rec_10_Q11	11,9	10,0	7,2		9,7	152,3	118,2	130,7		133,7
Pp	Rec_11_Q11	12,0	6,9	4,4		7,8	153,5	81,3	79,5		104,8
Pp	Rec_12_Q11	1,2	3,2	0,2		1,5	10,1	37,4	3,7		17,1
Pp	Rec_13_Q11	1,6	2,4	0,4		1,4	20,2	28,0	7,0		18,4
Pp	Rec_13'_Q11	3,3	1,4	0,1		1,6	27,5	16,8	0,9		15,1
Pp	Rec_14_Q11	2,4	0,8	0,7		1,3	31,1	8,9	12,6		17,5
Pp	Rec_15_Q11	0,6	2,1	0,5		1,1	5,0	24,3	9,2		12,8
Pp	Rec_16_Q11	0,8	0,9	0,3		0,7	7,0	10,8	5,1		7,6
Pp	Rec_17_Q11	1,9	2,0	0,9		1,6	23,8	23,5	16,3		21,2
Pp	Rec_18_Q11	1,3	3,3	1,3		2,0	16,9	39,2	23,4		26,5
Pp	Rec_19_Q11	1,4	1,8	0,3		1,2	12,0	21,3	5,6		13,0
Pp	Rec_20_Q11	3,3	6,8	2,5	3,9	4,1	27,6	80,4	44,7	67,2	55,0

The RIF is continuously and quantitatively distributed for all of the rusts; an example is given of *Pp* (Figure 14; rest rusts appendix figures 21). Importantly, the RIF has two peaks, one with resistant genotypes and one for susceptible genotypes. Overall, Steptoe and SusQ11 showed high resistance while SusPtrit was one of the most susceptible. Some lines were more susceptible than SusPtrit for some pathogens while others were more resistant than SusQ11. None of the recombinants were more resistant than Steptoe for any of the pathogens tested.

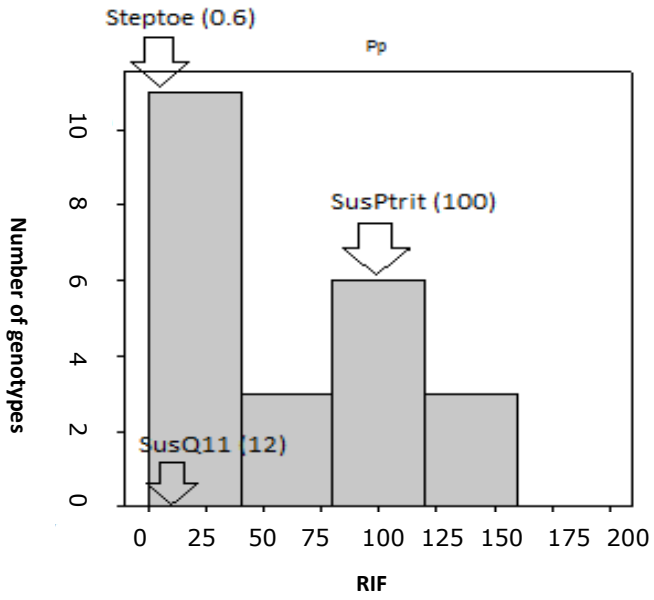


Figure 14. Frequency distribution of the average RIF for the pathogen *Pp*. The RIF values of the parental lines are indicated by an arrow, the exact value is shown between parentheses. Y-axis: number of genotypes; x-axis: RIF

Data of the genotyping and the phenotyping were used to perform two types of mapping procedures: (i) A QTL mapping procedure and (ii) a substitution mapping procedure, as described in the data analysis section. In the end, data of the phenotyping and genotyping of this study and data of studies performed by Yeo et al (unpublished), van Dijk (2007), Yeo (2007), Kuijken (2009) and Salunke (2013) we used for the purpose of fine mapping the *Phs*/*Phb* QTL and to determine whether more QTLs were present. Results are presented in the following paragraph.

3.1.3 QTL analysis

A single-marker QTL analysis was performed on several representative, evenly spread, markers across chromosome 2H. The same markers were used as in the study performed by Salunke (2013), in addition to the markers developed in this study (Table 8). Significance values of the analysis can be found in the appendix (§7.1.1). The effect of two main effects was tested, namely “allele” and “replicate” and, in addition, the interaction between the two. The model can be found in the data analysis section. For this study, the effect of allele was the most informative, as this indicates a significant difference in the mean for the allele of Steptoe (A) and the allele of SusPtrit (B). A significant difference indicates that the marker is associated with difference in RIF, and thus with the trait resistance to heterologous rust(s). One allele is then associated with higher RIF, the other with a lower RIF. The factor “replicate” was taken into the model, because a significant interaction can indicate a different effect of “allele” in different replicates; the so-called cross-over interaction. Significant interactions were found in the analysis (α=0.05), however the interaction appeared to be “divergent” or “convergent” (Figure 15). This indicated that the differences between the alleles differed in size between the replicates (Table 28-35, appendix) and that an additive model cannot be assumed directly. However, in the case of marker association, it was concluded that this not influenced the main effect “allele”. The interaction was therefore not taken into account during the later stages of the QTL analysis, only the main effect “allele”. The main effect “replicate” was not used in the analysis of the marker effect, as it did not have a significant effect in most of the cases.

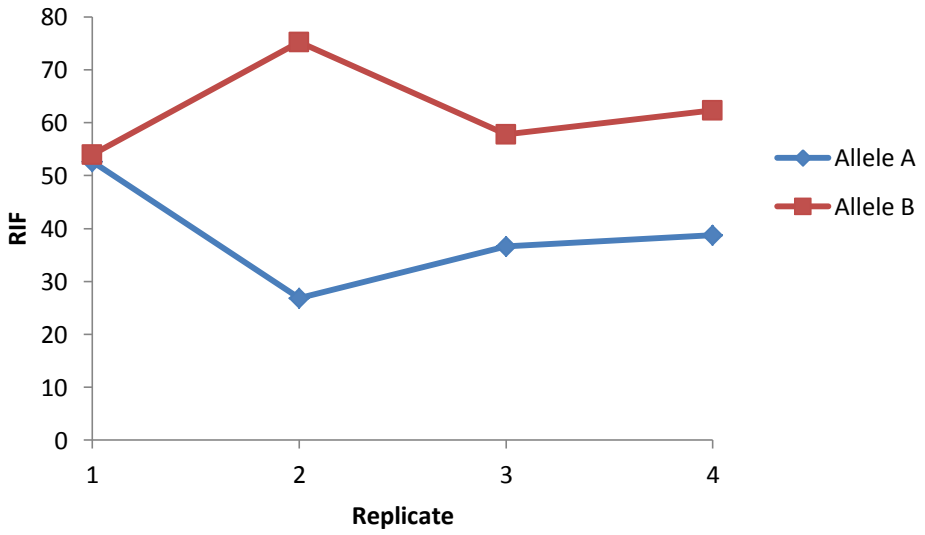
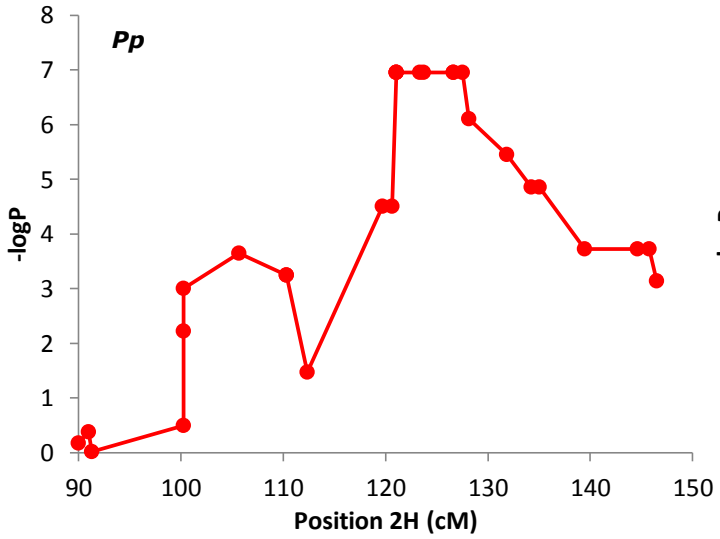


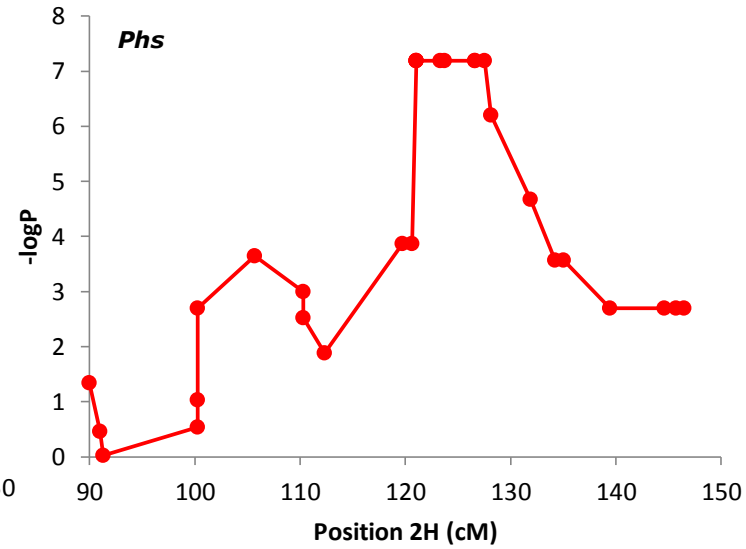
Figure 15. Example of an interaction for marker 57. In blue, the average RIF of allele A is indicated for each replicate. In red, the average RIF of allele B is indicated for each replicate. A divergent interaction can be observed between replicate 1 and 2; later the interaction becomes more convergent.

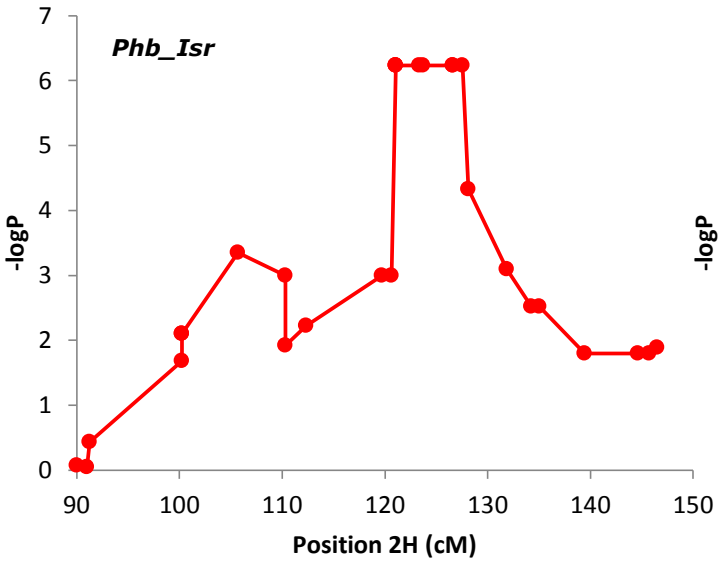
Results of the QTL analysis are displayed graphically in figure 16. For all of the rusts analyzed, the highest p-value was for the region 121.05cM to 127.53cM. The markers between the two flanking markers of this region showed the highest association between the RIF value; a clear peak in each of the graphs can be observed. Markers near the peak show a gradual increase/decrease in significance. The markers in that region are 25, 62, 64, 68, 69, 73, 74 and 75; most of which were specifically designed in this study to fine map that region. Marker 25 was designed by Salunke (2013). A smaller peak, somewhat less significant, is found for each of the rusts species at around 100-110cM. The A allele (Steptoe allele) was associated with a lower RIF than the B allele (SusPtrit allele) in most of the cases.

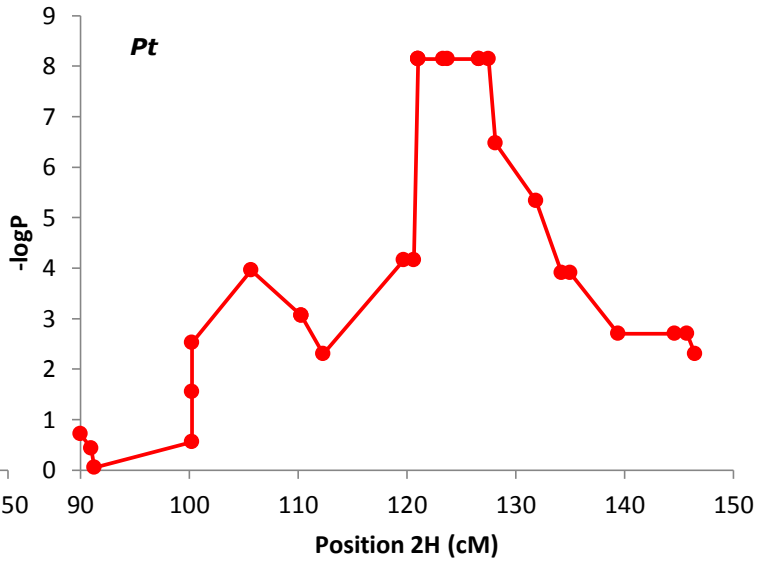
For the rust *Pgl*, one significant marker (WBE129, close to *Rphq11*) around 90cM (precise position not known) was found that showed a significant association with the RIF. However, in only this case, the SusPtrit allele was associated with a low RIF and the Steptoe allele was associated with a high RIF.

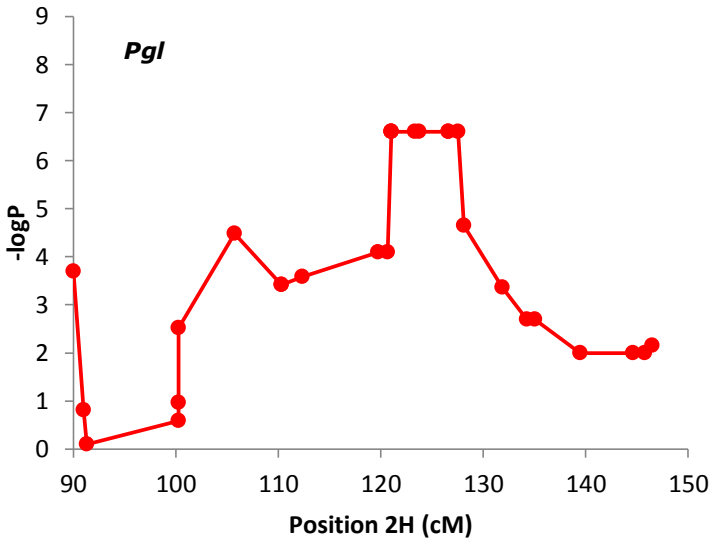
For the rust *Phb_Iran* an almost significant association (α=0.002) was found for marker WBE144 (Indicated at 90cM, precise position is not known however; Figure 16), which is the peak marker for *Rphq11*. This would coincide with the two-locus interaction found for *Phb_Iran*.

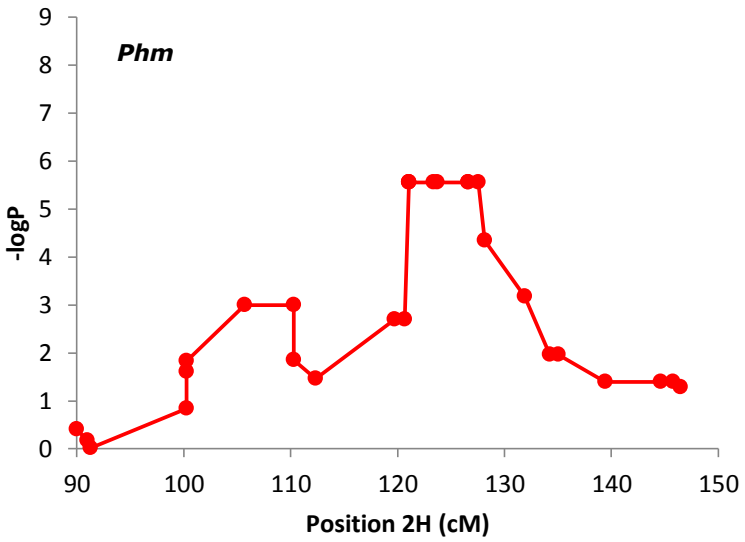

Pp
-logP
Position 2H (cM)


Phs
-logP
Position 2H (cM)


Phb_Isr
-logP
Position 2H (cM)


Pt
-logP
Position 2H (cM)


Pgl
-logP
Position 2H (cM)


Phm
-logP
Position 2H (cM)

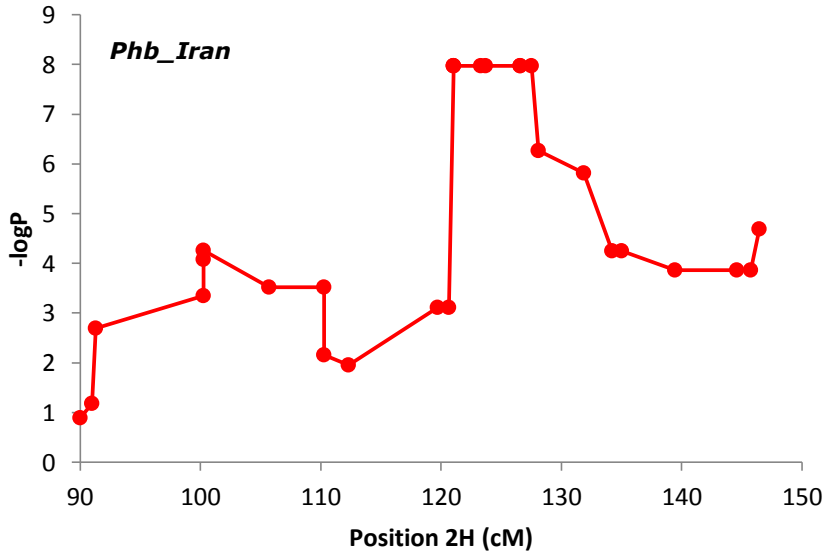


Figure 16. QTL mapping results. –logP (P=p-value ANOVA, appendix table 26-33) values are plotted against the position on chromosome 2H for each marker. This is done for all of the six tested heterologous rusts and for *Phb_Iran*, which was tested in previous studies (Yeo et al, unpublished). Higher values indicate a more significant marker association with the RIF. Example: a p-value of 0.001 has a –logP value of 3.

3.1.4 Substitution mapping

Results from the substitution mapping are presented in table 11. Analysis results can be found in the appendix (§7.1.4). Overall, genotypes were grouped with high significance to either a "susceptible" group containing allele "B" and to a "resistant" group containing allele "A" with the Dunnets post-hoc test. In some cases, RIF values were either higher than SusPtrit or lower than SusQ11. These cases were then still assigned to the susceptible and resistant group respectively due to the high effect of the QTL. Cases that were both significantly different, in terms of RIF, than SusPtrit and SusQ11 were grouped to a third group. In this case, no allele (A or B) could be assigned.

For most of the pathogens, the same pattern of susceptibility/resistance alleles is observed for all of the recombinants, i.e. recombinant 1 (Rec 1) is susceptible, recombinant 2 is resistant, recombinant 3 is again susceptible, recombinant 4 is again resistant, recombinant 5 to 11 are susceptible, recombinant 12 to 19 are resistant and lastly, recombinant 20 is susceptible. *Pgl* is the only pathogen where recombinant 4 is grouped to the susceptible class.

Results from table 11 were visually mapped to the marker map available of the region 2H (Yeo et al, unpublished. Martin-Sanz et al, unpublished (2013), Salunke (2013), van Dijk (2007), Kuijken (2009), Jafary et al. (2006b), Marcel et al. (2007b)) for the sub-NIL homorecombinants (Figure 17). Following the same procedure, *Rphq11* was mapped to peak marker WBE144 (Kuijken, 2009; van Dijk, 2007). The allele pattern for the pathogens *Phs*, *Phb_Isr*, *Pt*, *Phm* and *Phb_Iran* was identical to the pattern for the alleles in the region 121.05-127.53cM, i.e. marker 25, 62, 64, 68, 69, 73, 74 and 75. This was despite several un-assignable alleles for some recombinants. These alleles did not appear to be crucial for the mapping procedure.

The allele pattern for *Pgl* could not be associated with any marker in the region due to the SusPtrit allele of recombinant 4, which is significantly different from the Steptoe allele associated with other pathogens. However, recombinant 4 has been observed as having uncharacteristically small seeds (about ¼ size of other homorecombinants) and being poor germinating and slow growing plants. The RIF of recombinant 4 is therefore somewhat unreliable, as these plants have small leaves making

evaluation of the IF/RIF more difficult. Also, these plants have a reduced fitness, making them more susceptible to natural variations during handling. This would account for the high spread in RIF and often unassignable alleles (B/A) for the other heterologous rusts tested in this study.

The allele pattern of *Pp* was mapped between marker 8 (120.65) and marker 25 (121.05) in the study of Salunke (2013) (Figure 17). In that study, recombinant 20 was identified as having a Steptoe allele (A). However, by performing multiple phenotypic replicates during this study, the presence of this allele is questioned. Data analysis shows that recombinant 20 is neither significantly identical, in terms of RIF, to either Steptoe or SusPtrit. If this recombinant is not used in the substitution mapping, the only region where the pattern can be successfully mapped is 121-128cM. It is also surmised that, when observing the phenotypic data from table 10, that recombinant 20 is susceptible rather than resistant in contrast with Salunke (2013).

Table 11. Overview of the phenotyping and the substitution mapping results. Each genotyped is displayed with the average RIF calculated from the replicates performed in this study and the study of Salunke (2013). The average RIF is given for each heterologous rust tested. Each RIF is related to an allele from either Steptoe (A) and SusPtrit (B) in line with the substitution mapping procedure performed by Salunke (2013). Some recombinants were statistically different from SusPtrit and SusQ11; these are described as B/A. Colors indicate highest and lowest RIF for each pathogen, from dark green (lowest RIF) to dark red (highest RIF). One allele (*Pgl*, recombinant 4) is indicated in red as this allele deviates significantly with the allele association found for other pathogens. * data is unreliable due to high spread in IF values between replicates.

	Pathogen													
Genotype	*Pp*		*Phs*		*Phb_Isr*		*Pt*		*Pgl*		*Phm*		*Phb-Iran*	
SusPtrit	100.0	B	100.0	B	100.0	B	100.0	B	100.0	B	100.0	B	100	B
Steptoe	0.6	A	0.0	A	2.8	A	0.3	A	7.3	A	0.3	A	5	A
SusQ11	12.0	A	16.6	A	10.2	A	32.9	A	36.1	A	18.3	A	10.6	A
Rec 1	100.6	B	75.6	B	79.7	**B/A**	78.4	B	100.0	B	47.9	**B/A**	61.4	B
Rec 2	14.7	A	11.9	A	17.9	A	34.6	A	67.0	**B/A**	27.8	A	2.5	A
Rec 3	87.7	B	89.0	B	111.9	B	83.9	B	160.7	B	80.4	B	50.4	B
Rec 4	52.1	**B/A**	29.7	A	37.9	**B/A**	33.3	A	75.4	B	18.4	A	13.9	**B/A**
Rec 5	76.4	B	112.6	B	72.0	B	94.3	B	204.2	B	74.9	B	62.6	B
Rec 6	155.5	B	81.8	B	54.8	B	81.4	B	85.1	B	64.4	**B/A**	58.4	B
Rec 7	82.3	B	75.2	B	102.7	B	77.8	B	141.2	B	71.3	B	68.9	B
Rec 8	84.9	B	91.6	B	99.3	B	81.7	B	132.8	B	75.5	B	56.9	B
Rec 9	138.8	B	70.7	B	98.0	B	97.1	B	113.8	B	81.1	B	98.5	B
Rec 10	133.7	B	80.8	B	91.1	B	90.9	B	102.6	B	75.3	B	109.4	B
Rec 11	104.8	B	96.7	B	104.0	B	114.5	B	113.6	B	86.6	B	98.4	B
Rec 12	17.1	A	32.1	A	14.9	A	31.9	A	37.8	A	32.0	A	2.3	A
Rec 13	18.4	A	21.6	A	21.8	A	35.4	A	38.5	A	24.8	A	3.2	A
Rec 14	17.5	A	27.4	A	21.7	A	34.8	A	66.9	**B/A**	33.4	A	5.1	A
Rec 15	12.8	A	16.9	A	15.6	A	30.2	A	37.6	A	25.6	A	3.2	A
Rec 16	7.6	A	33.3	A	28.0	A	35.5	A	49.2	A	37.5	A	2.7	A
Rec 17	21.2	A	26.6	A	20.8	A	30.2	A	52.5	A	31.2	A	34.6	B*
Rec 18	26.5	A	25.3	A	44.6	**B/A**	32.8	A	67.7	A	37.0	A	25.2	B*
Rec 19	13.0	A	27.6	A	23.7	A	19.8	A	66.6	A	27.1	A	32.3	A*
Rec 20	55.0	**B/A**	114.0	B	113.7	B	89.2	B	117.9	B	79.5	B	99.2	B

Position								91.28	100.26							105.72	112-119	110.03	110.03	112.33
Marker								WBE144	TC168528	TC174372	GBM1062	TC12856	ABC18091-1	WBE130	K04002	53	GBMS244	57	56	61
Genotype	Pathogen *Pp*	*Phs*	*Phb_Isr*	*Phb_Iran*	*Pt*	*Pgl*	*Phm*	Rphq11								LS CAPS		LS CAPS	LS	LS
Steptoe	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A A	A	A A	A	A
SusP	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B B	B	B B	B	B
SusQ11	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A A	A	A A	A	A
Rec. 1	B	B	B/A	B	B	B	B/A	A	A	A	A	A	A	A	A	A A	B	B B	B	B
Rec. 2	A	A	A	A	A	B/A	A	A	A	A	A	A	A	A	B	B B	B	B B	B	B
Rec. 3	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B B	B	B B	B	B
Rec. 4	B/A	A	B/A	B/A	A	B	A	A	A	A	B	B	B	B	B	B B	B	A A	A	A
Rec. 5	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	B B	B	B B	B	B
Rec. 6	B	B	B	B	B	B	B/A	A	A	B	B	B	B	B	B	B B	B	B B	B	A
Rec. 7	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B B	B	B B	B	B
Rec. 8	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B B	B	B B	B	B
Rec. 9	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B B	B	B B	A	A
Rec. 10	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B B	B	B B	B	B
Rec. 11	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B B	B	B B	B	B
Rec. 12	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A A	A	A A	A	A
Rec. 13	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A A	A	A A	A	A
Rec. 14	A	A	A	A	A	B/A	A	A	A	A	A	A	A	A	A	A A	A	A A	A	A
Rec. 15	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A A	A	A A	A	A
Rec. 16	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A A	A	A A	A	A
Rec. 17	A	A	A	B*	A	A	A	B	B	A	A	A	A	A	A	A A	A	A A	A	A
Rec. 18	A	A	B/A	B*	A	A	A	B	B	B	A	A	A	A	A	A A	A	A A	A	A
Rec. 19	A	A	A	A*	A	A	A	B	B	B	B	B	B	A	A	A A	A	A A	A	A
Rec. 20	B/A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B B	A	A A	A	A
								Ph												

	119.71	120.65	121.05	121.05	121.05	123.34	123.72	126.62	126.62	127.53	128.13	131.87	134.23	135.02	139.45	144.62	145.74	146.48
	1	8	25	62	64	68	69	73	74	75	41	38	45	35	47	49	21	23
Genotype	LS	LS	LS CAPS	CAPS	CAPS	CAPS	LS CAPS	LS	LS CAPS	CAPS	LS CAPS	LS	LS	Seq.	Seq.	Seq.	LS CAPS	LS
Steptoe	A	A	A A	A	A	A	A A	A	A A	A	A A	A	A	A	A	A	A A	A
SusP	B	B	B B	B	B	B	B B	B	B B	B	B B	B	B	A	B	B	B B	B
SusQ11	A	A	A A	A	A	A	A A	A	A A	A	A A	A	A	A	A	A	A A	A
Rec. 1	B	B	B B	B	B	B	B B	B	B B	B	B B	B	B	B	B	B	B B	B
Rec. 2	A	A	A A	A	A	A	A A	A	A A	A	A A	A	A	A	A	A	A A	A
Rec. 3	B	B	B B	B	B	B	B B	B	B B	B	B B	B	B	B	B	B	B H	A
Rec. 4	A	A	A A	A	A	A	A A	A	A A	A	A A	A	B	B	B	B	B B	B
Rec. 5	B	B	B B	B	B	B	B B	B	B B	B	B B	B	B	B	B	B	B B	B
Rec. 6	B	B	B B	B	B	B	B B	B	B B	B	B B	B	B	B	B	B	B B	B
Rec. 7	B	B	B B	B	B	B	B B	B	B B	B	A A	A	A	A	A	A	A A	B
Rec. 8	B	B	B B	B	B	B	B B	B	B B	B	B B	A	A	A	A	A	A A	B
Rec. 9	A	A	B B	B	B	B	B B	B	B B	B	B B	B	B	B	B	B	B B	B
Rec. 10	B	B	B B	B	B	B	B B	B	B B	B	B B	B	B	B	B	B	B B	B
Rec. 11	B	B	B B	B	B	B	B B	B	B B	B	B B	B	B	B	B	B	B B	B
Rec. 12	A	A	A A	A	A	A	A A	A	A A	A	A A	A	A	A	A	A	A A	A
Rec. 13	A	A	A A	A	A	A	A A	A	A A	A	A A	A	A	A	A	A	A A	A
Rec. 14	A	A	A A	A	A	A	A A	A	A A	A	A A	A	A	A	A	A	A A	A
Rec. 15	A	A	A A	A	A	A	A A	A	A A	A	A A	A	A	A	A	A	A A	A
Rec. 16	A	A	A A	A	A	A	A A	A	A A	A	A A	A	A	A	A	A	A A	A
Rec. 17	A	A	A A	A	A	A	A A	A	A A	A	A A	A	A	A	B	B	B B	B
Rec. 18	A	A	A A	A	A	A	A A	A	A A	A	A A	A	A	A	A	A	A A	A
Rec. 19	A	A	A A	A	A	A	A A	A	A A	A	A A	A	A	A	A	A	A A	B
Rec. 20	A	A	B B	B	B	B	B B	B	B B	B	B B	B	B	B	B	B	B B	B
			Pp , Phs , Phb_Isr , Phb_Iran , Pt, Phm															

Figure 17. Results from the substitution mapping procedure. For each pathogen and marker, the allele pattern is given for the homorecombinants and parental lines. The peak marker for *Rphq11* (*Ph* resistance) is indicated in yellow. The peak markers for *Pp*, *Phs*, *Phb_Isr*, *Phb_Iran*, *Pt* and *Phm* are indicated with an orange bar. For each marker, the marker name and position (cM) is given. For some markers the genotyping method is given. An "A" indicates the Steptoe allele, a "B" indicates the SusPtrit allele, U indicates an unassignable allele. Marker 25 is the peak marker for *Phs*/*Phb* resistance as indicated by Salunke (2013). Abbreviations: LS = LightScanner, Seq. = sequencing. * data is unreliable due to high spread in IF values between replicates.

3.1.5 Two locus interaction

RIF results were averaged for all the replicates considering four possible allelic combinations with *Rphq11*. This was done for the pathogens *Pp*, *Phs*, *Phb_Isr*, *Pt*, *Pgl* and *Phm* (Table 12). For the pathogens *Pp*, *Phs*, *Phb_Isr*, *Pt* and *Phm*, there are visually two groups observed, namely the group AA/BA with low RIF, and the group AB/BB, with high RIF. Thus when a SusPtrit allele is present in the 121-128cM region, plants are susceptible. *Phb_Iran* is a notable exception. 4 distinct groups are observed that cannot be explained by only taking the alleles of the 121-128cM region into account. A clear effect of *Rphq11* is observed. The results for the pathogen *Pgl* show a somewhat higher average RIF for the AB group in comparison with the BB group (Table 12). This is in contrast with the rest of the pathogens.

Genotypes in which no allele could be reliable declared (Table 11) were not taken into account. RIF averages for the pathogens except *Phb_Iran* therefore differs somewhat from the data of Salunke (2013).

Table 12. Overview of the average RIF for each allele combination of *Rphq11* and the 121-128cM region, given for each pathogen. Colors indicate highest and lowest RIF for each pathogen, from dark green (lowest RIF) to dark red (highest RIF). The Steptoe allele is indicated with an "A", the SusPtrit allele with a "B".

Rphq11	121-128cM	*Pp*	*Phs*	*Phb_Isr*	*Phb_Iran*	*Pt*	*Pgl*	*Phm*
A	A	14.7	24.1	20.0	3.2	34.3	44.4	27.3
B	A	20.2	26.5	29.7	32.3	27.6	62.3	31.8
A	B	97.9	87.6	86.7	59.7	82.9	128.5	75.5
B	B	125.8	90.6	101.7	101.4	97.9	112.0	80.7

The results of the QTL analysis showed a weak, but significant association between marker 53 (105.72cM) and the phenotypic variation (Figure 16 – peak near 105cM). It was therefore investigated whether a two-locus interaction existed between this region and the 121-128cM region (Table 13). This was the only significant association which was found for all the heterologous rusts.

Table 13. Overview of the average RIF for each allele combination of marker 53 and the 121-128cM region, given for each pathogen. Colors indicate highest and lowest RIF for each pathogen, from dark green (lowest RIF) to dark red (highest RIF). The Steptoe allele is indicated with an "A", the SusPtrit allele with a "B". Empty spaces indicate absence of the allele group, e.g. there were no recombinants displaying allele A for marker 53 and allele B for the 121-128cM region for the pathogen *Phb_Isr* (Table 11).

M: 53	121-128cM	*Pp*	*Phs*	*Phb_Isr*	*Phb_Iran*	*Pt*	*Pgl*	*Phm*
A	A	16.6	24.8	20.8	8.5	32.1	51.1	29.7
B	A	14.7	20.8	17.9	2.5	34.0		23.1
A	B	100.6	75.6		61.4	78.4	100.0	
B	B	108.0	90.3	94.2	65.4	90.1	124.7	78.1

It is difficult to determine whether a two-locus interaction exist for *Pgl* due to absence of an allele combination (BA). Also, only one recombinant displayed the allele A for marker 53 and allele B for the 121-128cM region. This data is thus unreliable. For the rest of the pathogens two clear groups can be observed, influenced by the 121-128cM region. However, again some allele combinations are missing.

3.1.5 Histological assessment

Almost no necrotic cells, and thus no HR response, were observed near the infections. Therefore, the counted necrotic infections were summed with the non-necrotic infections. The statistical analysis could not be performed on separate groups.

Results from the histological assessment of the *Phb_Iran* infection is summarized in table 14. For the width of the EST infections and the proportion of EA infections, three groups are observed, namely (1) Steptoe, (2) SusQ11 and homorecombinant 19 and (3) homorecombinant 7 and SusPtrit. The average width of EST infection is the largest in SusPtrit and homorecombinant 7, and the smallest in Steptoe. The proportion of EA infections is the largest in Steptoe and the smallest in recombinant 7 and SusPtrit.

Table 14. Average width (µm) of an established infection (EST), average proportion of early aborted (EA) infections (%), average IF and average proportion of EST infections containing sporogenic tissue (%) of several barley genotypes. Different letters indicate significant difference according to Fisher's protected LSD, with α=0.05. QTL alleles are shown in the column "genotype" where the first allele indicates the *Rphq11* allele and the second the *Phs/Phb* QTL allele.

Name	Genotype	Width (µm)	Prop. EA (%)	RIF	Sporogenic (% EST)
Steptoe	A - A	2.88a	72a	5a	0a
SusQ11	A - A	3.86b	40b	10.6b	0a
Recombinant 19	B - A	4.21b	43b	12.2b	0.5a
Recombinant 7	A - B	6.52c	23.5c	62.5c	6.75b
SusPtrit	B - B	7.16c	22c	100d	19c

In term of IF, different groups are observed, in which the average IF of Steptoe is the smallest and the average IF of SusPtrit the largest. The RIF of recombinant 7 is significantly different from SusPtrit, in contrast with the width of EST and the proportion of EA. Apparently the significantly larger infections and higher proportion of EST infections (in comparison with SusQ11 and recombinant 19) for recombinant did result in a higher RIF, but not as high as SusPtrit. Contrastingly, the width and %EA was the same as SusPtrit. SusQ11 and recombinant 19 show a low average RIF.
Almost no EST infections in Steptoe, SusQ11 and recombinant 19 contained sporogenic tissue. The number of EST infections containing sporogenic tissue was the largest for SusPtrit. Some EST infections of recombinant 7 contained sporogenic tissue, but significantly less than SusPtrit.

The histological assessment (Table 14) showed that in terms of %EA and width of the established infections, two groups are observed (not taking Steptoe into account, as Steptoe contains additional NHR QTLs) namely SusQ11 & recombinant 19 and SusPtrit & recombinant 7. It seems that this difference is associated with the *Phs/Phb* QTL and not *Rphq11*; differences in *Rphq11* do not create additional groups for these two components. The RIF is significantly different between SusPtrit and recombinant 7 (as mentioned above). In this case, the difference can be associated with the allele of *Rphq11*. The number of EST infections containing sporogenic tissue is significantly more in SusPtrit, which would account for the difference in RIF, i.e. it is the amount of counted pustules which determines the RIF.

In SusPtrit and recombinant 7 and SusPtrit, a great number of large infections can be observed whereas these numbers in SusQ11, Steptoe and recombinant 19 are clearly lower and the colonies are smaller (Figure 18). Also, no sporogenic (red) tissue is observed in those latter genotypes.

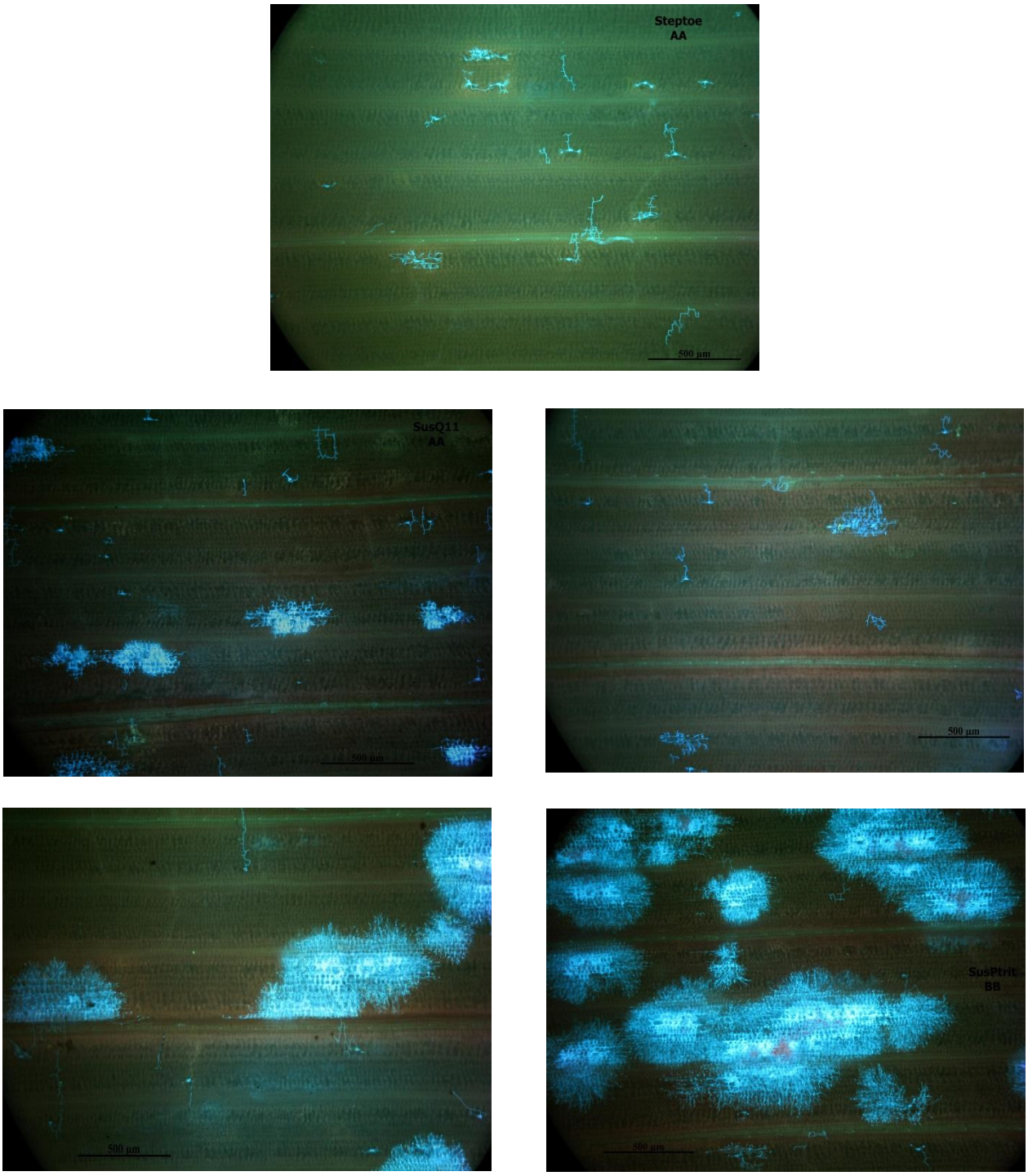


Figure 18. Photographs of leaf samples of Steptoe, SusQ11 (1st row left), homorecombinant 19 (1st row right), homorecombinant 7 (2nd row left) and SusPtrit (2nd row right), as viewed with a UV microscope (10x magnification, 1.0x zoom), with the rust fungi stained blue. Sporogenic tissue is colored red. The size of the infection is indicated by a scale bar. It should be noted that only a small part of the leaf is displayed. Pictures are only for illustrating the difference between susceptible and resistant genotypes.

3.2 Resistance to *Pp* and *Phb* in L94 background

3.2.1 Genotyping

Markers 3, 7, 8, 13, 26 and 36 were run on the 1501-2 to 1501-5 progeny (Table 15, primers Table 45). Every marker except marker 36 produced a clear, one band, amplicon. Marker 36 was therefore discarded from the pipeline. Genotyping was possible to do for marker 3 and 26 in the mapping population with the LightScanner procedure. Despite the parental lines for markers 7 and 13 were easily genotyped with Lightscanner (dominant markers with this method); it was not possible to have a clear interpretation in the mapping population. Marker 8 was run with the CAPs procedure (both enzymes), but no polymorphism was detected using this method (Figure 19).

Table 15. List of the used SNP markers with their respective: (i) locus name on the 9K i-select Infinium array, (ii) association with resistance to this specific rusts species (Chisenga, 2013) (iii) blast result of the array sequence, (iv) blast results of PCR amplicon sequence, (v) location on chromosome, (vi) location on the consensus map (CM), (vii) amount of PCR products, (viii) number of SNPs in the sequence of the PCR product, (ix) indication whether the markers were polymorphic (PM) in the parental lines (PL) with LightScanner, (x) indication if the markers were genotyped in the mapping population (MP) with LightScanner (Yes=genotyped), (xi) CAPs candidate of the PCR sequence. Blank cells indicate either absence of product, no polymorphism or no CAPs candidate. Parental samples of marker 3, 8, 13 and 26 were not sequenced during this study.

Marker	(i) Rust	(ii) Locus	(iii) Blast (array)	(iv) Blast (seq.)	(v) Chr.	(vi) Map Position cM	(vii) PCR amplification	(viii) Number SNPs	(ix) LS PL	(x) LS MP	(xi) CAPs	CAPs MP
3	***Pp***	**BOPA1_868-675**	AK248625.1		2H	123.338	1 band		PM	Yes		
8	***Pp/Phb_Isr***	**BOPA1_1381-547**	AK374555.1		2H	132.302	1 band	1			AccI, MwoI	
13	***Phb_Isr Pp***	**SCRI_RS_225187**	AK359036.1		5H	10.620	1 band					
26		**SCRI_RS_169728**	AK249894.1		6H	60.634	1 band		PM	Yes	Fnu4H, TseI	
36	***Pp***	**SCRI_RS_130605**	AK248406.1	AK248406.1	6H	60.634		1			PstI	

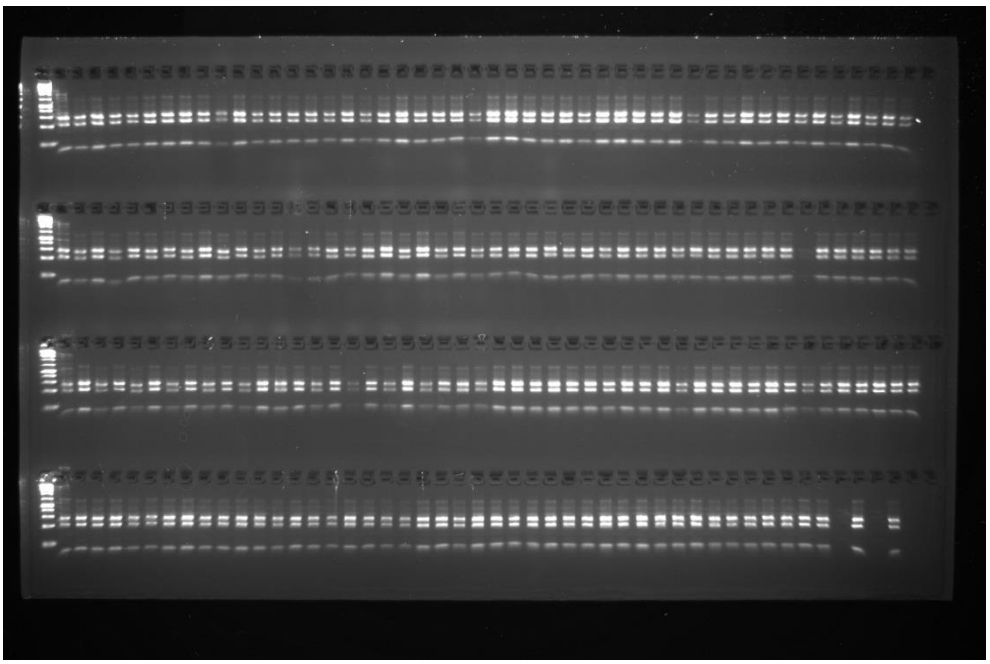


Figure 19. Picture of gel showing digested amplicons for marker 8, run on the 173 individuals of the 1501-2 to 1501-5 progeny. Banding pattern is identical for all the individual plants; no polymorphism is detected. Samples are not labelled in this picture.

3.2.2 Phenotyping

An example of the phenotyping results is displayed in Table 16 (rest, table 48-51). Results indicate that the progeny shows resistance and susceptibility in different levels (quantitative resistance). SusPtrit is very susceptible and L94 is almost completely resistant. Vada shows resistance, although it is not completely resistant as L94. None of the recombinants is as susceptible as SusPtrit and none is as resistant as L94.

Table 16. Example of the phenotypic results of inoculation with the pathogen *Pp* for progeny of 1501-5 and the parental and control lines. Colors indicate highest and lowest IF and RIF values, from dark green (lowest) to dark red (highest). The IF is calculated per cm^2 leaf area. The entire dataset, for this inoculation, is given in table 50. Plant number starts at 15, as plant 1-14 was phenotyped at another point during the study. Plant 26 died and was therefore not phenotyped.

Pathogen	Genotype	Plant number	IF	RIF
Pp	Vada	1	0.1	1.2
Pp	Vada	2	0.5	5.6
Pp	Vada	3	1.1	11.2
Pp	Vada	4	0.0	0.0
Pp	L94	1	0.0	0.0
Pp	L94	2	0.1	0.8
Pp	L94	3	0.0	0.0
Pp	L94	4	0.0	0.0
Pp	SusP	1	9.5	99.2
Pp	SusP	2	10.6	110.7
Pp	SusP	3	8.6	90.1
Pp	1501-5	15	0.6	6.5
Pp	1501-5	16	1.5	15.5
Pp	1501-5	17	0.9	9.1
Pp	1501-5	18	2.7	28.5
Pp	1501-5	19	3.3	34.2
Pp	1501-5	20	1.7	18.0
Pp	1501-5	21	0.5	5.3
Pp	1501-5	22	4.2	43.6
Pp	1501-5	23	5.9	61.8
Pp	1501-5	24	2.3	24.0
Pp	1501-5	25	3.2	33.6
Pp	1501-5	26	x	x
Pp	1501-5	27	0.9	9.0
Pp	1501-5	28	1.8	19.2
Pp	1501-5	29	1.5	15.6
Pp	1501-5	30	5.0	52.3

3.2.3 Marker analysis

The two genotyped markers are, according to Chisenga (2013), associated with *Pp* resistance. No markers were genotyped that were associated with *Phb_Isr* resistance, according to Chisenga (2013). Results of the chi-square test showed that marker 3 segregated according to Mendelian ratios (p = 0.391) and that marker 26 deviated significantly (p = 9.55* 10^{-5}) from the 1:2:1 ratio (co-dominant markers) (Table 17). This is most notably seen in the amount of SusPtrit genotypes scored for marker 26, which is much less than expected, i.e. only 16 individuals are observed were 39 are expected. This distorted segregation was not found by Chisenga (2013).

Table 17. Chi-square table indicating the observed (Obs.) and expected (Exp.) amount of progeny plants displaying a certain genotype, for marker 3 (left table) and marker 26 (right table). These markers are associated with *Pp* resistance. Marker 26 deviated significantly from a 1:2:1 ratio (chi-square test), whereas marker 3 does not. Abbreviations: L = L94, H = heterozygous, S = SusPtrit, M = marker. The alleles of some genotypes were unassignable and are therefore not displayed.

M: 3	L	H	S	Total
Obs.	42	65	39	146
Exp.	36.5	73	36.5	146

M: 26	L	H	S	Total
Obs.	50	90	16	156
Exp.	39	78	39	156

For marker 3 and 26 it was tested whether there was a significant association between the average RIF and the scored genotypes of the 1501-2 to 1501-5 progeny. Results show that markers 3 and 26 are not associated with the RIF of *Phb_Isr*, as was expected (Table 18, first row). For each of the genotypes, the average RIF is not significantly different. The average RIF for *Pp* is significantly different for the genotypes; three distinct groups are observed where the group homozygous for the L94 allele has the lowest RIF (most resistant) and the group homozygous for the SusPtrit allele has the highest RIF (most susceptible). The heterozygous group has an intermediate RIF. For marker 26, a significant difference was found for the average RIF of *Pp* where the L94 group has the lowest RIF and the H & S group the highest. The heterozygous group and SusPtrit group are not significantly different.

Table 18. ANOVA and LSD results (α = 0.05) showing the average RIF per marker allele. These markers are associated with *Pp* resistance. Alleles s are indicated with a letter. Abbreviations: L = L94, H = heterozygous, S = SusPtrit. M = marker. First row, average RIF of *Phb_Isr*; second row, average RIF of *Pp*.

Allele marker 3	RIF *Phb_Isr*	Groups
L	11.88	a
H	23.85	a
S	29.06	a

Allele marker 26	RIF *Phb_Isr*	Groups
L	24.89	a
H	20.74	a
S	29.65	a

Allele marker 3	RIF *Pp*	Groups
L	8.816	a
H	22.55	b
S	34.89	c

Allele marker 26	RIF *Pp*	Groups
L	13.14	a
H	24.14	b
S	38.74	b

Table 19 shows an overview of the allelic combinations of marker 3 and 26, and their association with the RIF of *Pp*. An interesting result is the average RIF of the plants containing both SusPtrit

alleles for the markers. The average RIF is 45, whereas the average RIF of SusPtrit is 100. The RIF of genotypes homozygous for the L94 allele for both markers are more similar to the L94 phenotype, confirming thus the effect of the QTLs at markers 3 and 26. Other combinations of the alleles show an intermediate phenotype. Marker 3 seems to be the one with major effect in explaining the resistance. This data corroborates the previous research done by Chisenga (2013). This data also support the additive effect of the resistance QTLs.

Table 19. Allelic combinations for marker 3 and 26 and the average RIF for *Pp* for each combination. The average RIF is sorted from lowest to highest. Abbreviations: L = L94, H = heterozygous, S = SusPtrit. The "x" indicates that genotypes containing the L – S genotype were not found in this study.

Marker 3	Marker 26	RIF
L	L (L94)	0
L	L	3.955
L	H	13.56
S	L	17.88
H	L	19.97
H	S	22.15
H	H	23.86
S	H	42.31
S	S	45.37
S	S (SusPtrit)	100
L	S	x

4. Discussion

During this MSc thesis project a fine mapping procedure of the introgression of SusQ11 was continued and a mapping procedure on a relatively new resistance gene (locus) of L94 was continued.

SusQ11

In this study, seven new markers were developed in the 121-128cM region of the introgression in SusQ11. Steptoe and SusPtrit alleles identified in the homorecombinants were identical for these seven markers and the peak marker 25 (*Phs*/*Phb* QTL, as identified by Salunke (2013)). Using two methods, QTL mapping and substitution mapping, resistance to *Pp*, *Phs*, *Pt*, *Phm*, *Phb_Isr* and *Phb_Iran* was mapped to the seven markers and marker 25. Resistance was thus mapped between the flanking markers of this region (120.65 and 128.13cM). This region will be called hereinafter *Rnhq121cM* (as Rust NonHost resistance Qtl 121cM).

In other mapping populations used in previous studies, resistance to several of the pathogens used in the current study was found (Table 20). The resistance QTLs found in VxS, L94xS and GPxS could be the same as the QTLs found for resistance from Steptoe in this study, as these QTLs are located at almost the same location and are effective against the same heterologous rust(s). This is only speculation at this point.

Table 20. Overview of barley resistance QTLs detected in the interval 90-140cM, as described by several studies. The (i) array locus, (ii) peak marker, (iii) linkage group (LG), (iv) location on the consensus map (CM), (v) mapping population, (vi) resistance to which pathogen species, (vii) the QTL donor, (viii) the QTL name and (ix) study in which the QTL was described, is given for each QTL.

(i) Locus	(ii) Other peak markers	(iii) LG	(iv) cM	(v) Population	(vi) Pathogen species	(vii) Donor	(viii) QTL Name
BOPA2_12_10969	MWG503	2H	**91.283**	SxM	*Ph*	Steptoe	*Rphq11*
E37M33-162		2H	**95.123**	CCxS	*Ph*	Cebada Capa	*Rphq11*
BOPA1_8523-316	E32M61-174	2H	**108.178**	VxS	*Phb_Isr*	Vada	
SCRI_RS_154703		2H	**121.684**	GPxS	*Pt*/*Pp*	Golden Promise	
BOPA1_868-675	E33M61-227	2H	**123.338**	VxS/L94xS	*Phb_Isr*/*Pp*	SusPtrit/L94	
SCRI_RS_156045		2H	**124.508**	GPxS	*Phs*	Golden Promise	
BOPA1_1381-547		2H	**132.302**	L94xS	*Pp*/*Phb_Isr*	L94	

Unfortunately, no recombinations were detected between marker 25 and marker 41 (Table 9; Figure 17); recombinations are key in further fine mapping the region. For future fine mapping purposes, crossing of e.g. SusPtrit and SusQ11 would allow recombinations for the entire introgression. Selection using the flanking KASPar markers, constructed in this study, during the F_2 would identify progeny with recombinations between marker 7 and marker 47, and thus possibly in *Rnhq121cM*. These KASPar markers are allele specific, making it possible to select for the presence/absence of a

Steptoe, SusPtrit or heterozygous allele at either flanking points of a QTL. Another method for the construction of recombinants would be selfing of the heterozygous recombinants, which is an earlier backcross generation (BC_5S_1). This would also allow for recombination across the entire introgression.
The resistance to *Pgl* was only mapped by the QTL mapping procedure; it was surmised that this method uses the average difference in RIF between Steptoe and SusPtrit alleles and not the presence of a Steptoe or SusPtrit allele in absolute terms; as is done in the substitution mapping procedure. The implied difference in the results of the two techniques is the result of recombinant 4, which shows susceptibility to *Pgl*. Visual mapping is not possible then to any of the markers of chromosome 2H available in this study. However, as explained in §3.1.3, RIF results for recombinant 4 are somewhat unreliable. It is possible that *Pgl* can be mapped to *Rnhq121cM*. An additional round of phenotyping with recombinant 4, using more plants, and the future map-based cloning can be used to confirm this. For *Phb_Iran* however, a failure of visual mapping using only one locus resulted in the identification of additional genes influencing resistance (two-locus interaction, Niks, R.E. personal comment). This could also be the case here, for *Pgl*. This was investigated for marker 53 (Table 13), however data was too unreliable to draw unambiguous conclusions. The presence of additional loci should be investigated in future studies not only for *Pgl*, but for the other rusts as well, and should be performed after the construction of additional recombinants as more recombinants are needed displaying specific combinations of alleles (Table 13).

Observing the sums of squares of the QTL analysis approach, markers (genotypes) explained about 25-40% of the RIF variation (data not shown). Although replication effects and interaction effects (Table 28 – 35) explained some variation, often more than 50% of the variation is identified as residual. At this point it is unclear whether other (minor) QTLs are present on the introgression or whether the variation can mostly be explained by natural variation from sources such as plant handling, climate effects etc.

Phenotyping results show a quantitative distribution of the RIF for all of heterologous rusts used in this study. In addition, these distribution has roughly two peaks, one for the susceptible genotypes and one for resistant genotypes (Figure 14). Steptoe shows in all cases significantly ($p = 2.96*10^{-8}$) the most resistance, which was expected as this line contains additional QTLs not present on the introgression of SusQ11 (Jafary et al., 2008). However, in not all of the cases SusPtrit is on average the most resistant. For example for the rust *Pp* (Table 10), recombinant 10 shows a higher RIF in all of the replications. However, this is not significant and is likely due to natural variation. The Steptoe allele of marker WBE129 and TC161220-1 shows a pattern of association with susceptibility to *Pgl* (Figure 16, Table 28-35); i.e. the Steptoe allele is present in individuals with a high RIF, whereas the SusPtrit is present in individuals with a low RIF. This association is significant for the pathogen *Pgl*. However, this cannot be confirmed by means of the substitution mapping analysis as recombinants containing a RIF higher or the same as SusPtrit were given a susceptibility allele; a method which implies SusPtrit being the susceptible parent. In this case, this process is unreliable as genotypes containing the SusPtrit allele are apparently more resistant.
A two locus interaction was observed for *Phb_Iran* (Table 12). Four groups in terms of RIF were found that were visually associated with the presence of either a SusPtrit/Steptoe allele for the two QTLs (Salunke, 2013).

The histological data collected in this study suggest that *Rnhq121cM* influences the amount of EA and width of pustules to a high extent whereas *Rphq11* influences the speed of the infection to a smaller extent (Table 14). This two locus interaction has then more an additive nature, were *Rphq11* contribute to the disease resistance of *Rnhq121cM*, conferring resistance. The SusPtrit *Rphq11* allele does not seem to result in susceptibility, as there is no significant difference between recombinant 19 and SusQ11 in terms of RIF, %EA, width and percentage of sporogenic tissue. This in in contrast with data from table 12, which indicated and relatively susceptible phenotype in genotypes containing only the *Rphq11* SusPtrit allele.

For most of the other heterologous rusts tested in this study only two groups were observed, either susceptible or resistant as explained by the QTL *Rnhq121cM*. However, the pathogens *Pp*, *Phb_Isr* and *Phm* showed a weak trend with *Rphq11*, where the BA group was less resistant than the AA group and where the AB group was less susceptible than the BB group. This would imply a smaller two locus interaction than for *Phb_Iran*. Using an ANOVA and determining whether the four groups are significantly different can help determining this interaction and account for variation in future studies. In addition, other loci can interact with *Rnhq121cM* (see above).

Almost none of the cells were observed to be necrotic. Although NHR responses have been mostly associated with a pre-haustorial reaction (non-HR), a HR response has been observed in the heterologous rust *P. triticina* (Niks, 1983a). Apparently, resistance to *Phb_Iran* is not associated with a HR response.

Resistance to Pp and Phb_isr in L94 background

Chisenga (2013) identified three QTLs associated with *Pp* resistance and two QTLs associated with *Phb_Isr* resistance. The QTLs for *Pp* resistance were associated with marker 3, 8 and 26 and the QTLs for resistance to *Phb_Isr* were associated with marker 8 and 13. Only marker 3 and 26 were useful in genotyping the 1501-2 to 1501-5 progeny in this study. Polymorphisms were not detectable for marker 7, 8, 13 and 36 with LightScanner and/or CAPs. This was in contrast with previous studies by Chisenga (2013) and Alberto Martin Sanz (unpublished), in which it was possible to do the genotyping with the LIghtScanner procedure. Markers 7 and 13 were found the be relatively easy to genotype, because they behave as dominant markers in the LightScanner. Why the genotyping failed in this study is unclear at this point, but genotyping with the LightScanner is not always easy and depends on the quality of the PCRs, the person doing the evaluation and other factors. For identification of the associated markers in the progeny, it would be better to genotype with all the markers and therefore redo the genotyping process. When that fails, the next step would be to develop primers from other SNP loci close associated with resistance, and which are close to markers 3, 7/8, 13 and 25/26/36. These polymorphic SNPs (between L94 and SusPtrit) can be found using the array, the consensus map of the group and the BSA performed by Chisenga (2013). The ideal new markers should have clear CAPs candidates to facilitate the genotyping (figure 20).

	Population						Genotype			Consensus map		Array		OWB				
Locus	G092814R	G092814S	G092816R	G092816S	G092817R	G092817S	L94	SusPtrit	Vada	LG	cM	i-select_LG	i-select_Pos	OWB_LG	OWB_Pos	CAPs candidates	(mega)BLAST	SNP (bp)
BOPA1_871-462	G	G	G	G	G	G	A	G	A	2H	123.34	2H	113.48	2H	132.56	HaeIII, NlaIV	EF514912.1	302
BOPA1_868-675	M	M	A	A	C	A	C	A	C	2H	123.34	2H	113.48	2H	132.56			
BOPA2_12_21396	R	R	A	A	R	A	G	A	G	2H	124.51	2H	113.92					
SCRI_RS_158091	C	failed	T	T	C	T	C	T	C	2H	126.08	2H	107.93			FnuDII (BstUI)	AK250555.1	192
SCRI_RS_149429	Y	Y	T	T	failed	T	C	T	T	2H	128.13	2H	112.04			NlaIV	AK353879.1	951
SCRI_RS_206070	M	M	C	C	failed	C	A	C	C	2H	131.03	2H	110.20			SphI	AK356041.1	1246
SCRI_RS_205571	Y	Y	C	C	T	C	T	C	C	2H	131.87	2H	112.32					
BOPA1_1381-547	R	R	A	A	G	A	G	A	G	2H	132.30	2H	121.50					
SCRI_RS_173490	R	R	G	G	failed	R	A	G	A	2H	132.69	2H	113.88					
SCRI_RS_132586	Y	Y	T	T	failed	T	C	T	T	2H	133.03	2H	112.04			CviJI	AK357586.1	1928
SCRI_RS_151556	Y	Y	C	C	T	C	T	C	C	2H	133.03	2H	112.18			Hpy178III	AK370427.1	525
BOPA2_12_20257	R	R	R	R	G	G	A	G	A	5H	1.95	5H	2.22			SpeI	AK250551.1	1264
BOPA2_12_30591	S	S	S	S	C	C	G	C	G	5H	5.30	5H	5.68			Hpy99I	AK365864.1	3423
BOPA2_12_31094	R	R	R	R	A	A	G	A	G	5H	10.62	5H	8.61	5H	25.65			
SCRI_RS_181570	failed	Y	Y	Y	T	T	C	T	C	5H	10.62	5H	8.61					
SCRI_RS_225187	A	R	failed	R	G	G	failed	G	A	5H	10.62	5H	8.61					
PA1_ABC08769-1-1-2	A	A	C	A	M	M	C	A	C	6H	60.08	6H	50.07	6H	61.25			
SCRI_RS_125353	G	G	A	G	R	R	A	G	A	6H	60.08	6H	50.07					
SCRI_RS_170058	A	A	G	A	R	R	G	A	G	6H	60.27	6H	52.48			HpaII	DQ245943.1	Not found
SCRI_RS_182195	A	A	C	A	M	M	C	A	C	6H	60.27	6H	52.83			TaqI	AK361930.1	Not found
)PA1_ABC04676-1-1-	T	T	A	T	W	W	A	T	A	6H	60.27	6H	53.29	6H	64.96			
SCRI_RS_188523	C	C	G	C	S	S	G	C	G	6H	60.31	6H	52.90					
BOPA2_12_30441	A	A	G	A	R	R	G	A	G	6H	60.52	6H	54.60					
SCRI_RS_232893	A	A	G	A	R	R	G	A	G	6H	60.57	6H	50.85					
SCRI_RS_9980	C	C	T	C	Y	Y	T	C	T	6H	60.57	6H	50.85					
SCRI_RS_159136	T	T	C	T	Y	Y	C	T	C	6H	60.59	6H	52.48			HhaI	AK365923.1	634
SCRI_RS_136897	G	G	A	G	R	R	A	G	A	6H	60.59	6H	52.76			BclI	No data	
SCRI_RS_161167	G	G	A	G	R	R	A	G	A	6H	60.63	6H	52.48					
SCRI_RS_130605	A	A	G	A	R	R	G	A	G	6H	60.63	6H	52.48					
SCRI_RS_169728	C	C	T	C	Y	Y	T	C	T	6H	60.63	6H	52.90					
BOPA2_12_31007	G	G	A	G	R	R	A	G	A	6H	60.65	6H	54.60	6H	64.96			

Figure 21. Overview of markers associated with the different BC3-S2 populations and parental genotypes in the BSA of Chisenga (2013). In that study a BSA was performed on several BC3-S2 barley populations, from the cross between L94xSusPtrit, namely: G092814, G092816 and G092817 and on control/parental lines (Vada, SusPtrit and L94). These populations were divided into plants displaying a resistant phenotype (denoted with "R") and plants displaying a susceptible phenotype ("S") to *Pp* and *Phb_Isr*. The different alleles of the displayed markers were found the be associated with either an susceptible or resistant population or with different control/parental lines, and can thus be located near QTLs for resistance to *Pp* and/or *Phb_Isr*. For each marker the linkage group and position on the consensus map, i-select array and Oregon Wolf Barley is indicated. In addition, for each marker a CAPs candidate is indicated which can be used in genotyping. The blast result from the array sequence and the position of the SNP in the found sequence is also given. The column "Locus" indicate the locus name on the 9K i-select Infinium array. Colours in this column indicate: Red = markers used in this study; white = other markers associated with resistance, but without CAPs candidate; Green = Markes associated with resistance with CAPs candidate; Purple = Marker in the same position as one associated with resistance, but with different results in the bulks; Orange = Restriction enzymes advised for cleavage of PCR products. Single letters indicate the different alleles. Y = pyrimidine, R = purine, M = cytorsine or adenine, S = cytosine or guanine, W = thymine, adenine or uracil, A = adenine, G = guanine, C = cytosine and T = thymine.

Data of this study suggest an additive association of the L94 allele with *Pp* resistance for marker 3, where individuals homozygous for the L94 allele have the lowest RIF, heterozygous individuals an intermediate RIF and individuals containing the SusPtrit allele have the higher RIF. Marker 26 is also associated with resistance, individuals homozygous for the L94 allele have the lowest RIF and both the heterozygous and the SusPtrit allele plants have the higher RIF. It was expected that the H and S groups would be different as this was found by Chisenga (2013), but the spread of the data is so large that this is not the case. This large spread could be the result of unassignable alleles. Therefore, a trend of an additive effect for the L94 allele is observed. However, one could also argue that the SusPtrit allele is dominant and susceptibility is therefore observed in the heterozygous progeny. Future genotyping and phenotyping as described above are needed to clearly establish the gene effect. Moreover, progenies of almost all the phenotyped and genotyped plants were obtained during this project.
None of the marker, genotyped in this study, is associated with *Phb_Isr* resistance. Data thus corroborates the results of the study of Chisenga (2013).

Marker 26 (n=156) showed a distorted segregation (Table 17), i.e. genotypic frequencies are skewed from their Mendelian expectations. The number of progeny identified as having one SusPtrit allele is significantly lower than expected. Segregation distortion can have numerous reasons (pollen tube growth, offspring death etc. (Lu et al., 2002)) and is also observed in barley (Devaux et al., 1995). However, genotyping with LightScanner is a subjective method and errors could have arisen there. Concurrently, some melting curves did not allow reliable genotyping; these individuals were marked "U" (unassignable) (Table 46 + 47). Possibly, the distorted segregation is the result of this erroneous or "incomplete" genotyping. If all the unassignable alleles where SusPtrit, then the segregation would not be distorted. Additional genotyping would be needed to confirm this. This distortion was not present in the study performed by Chisenga (2013). Although population sizes where smaller (n = 33, 74 and 64; three populations), no trend was observed towards a distorted segregation (p = >>0.05) in this study.
If the genotyping result from this study are replicated using the markers described in Figure 20, then strategic progeny could be sequenced for marker 8. Several plants, for example, containing the SusPtrit allele for both marker 3 and 26 could be sequenced and plants identified that only contain the marker 8 QTL. Phenotyping would then determine whether the QTL located at 2H; 132.cM is a major or minor effect QTL for both *Pp* and *Phb_Isr* resistance. Efforts could then be undertaken to sequence marker 13 in strategic progeny with different combinations of markers.. Marker 13 was not found to be polymorphic in this study, in contrast with Chisenga (2013).
By means of association of the RIF and allelic combination (Table 19) it is suggested that the largest effect QTL is located at the position of marker 3. Progeny possessing the L94 allele at this locus have on RIF reduction of 30% (Table 19), in comparison with individuals possessing the L94 for marker 26 (Table 19). Also, possessing the SusPtrit allele leads to a somewhat higher RIF, as is shown in the difference of S-H and H-S.
Genotypes containing both the SusPtrit alleles for the marker 3 and 26 locus do not display on average a RIF of 100; the RIF value of SusPtrit. Therefore, not all the variation in RIF is explained by both of the markers, suggesting an influence of additional genes. This is also suggested by Chisenga (2013), where an association with marker 8 is proposed; a marker which was not genotyped in this study. Additionally, the chi-square test suggests a distorted segregation for marker 3 and 26. If this is due to genotyping errors and not segregating distortion by an inherent characteristic of the

chromosomal region, this could lead to more of the contrast explained when a Mendelian segregation is taken into account. More genotyping with the specific markers used in this study and the study of Chisenga (2013), as described above, is needed to confirm this.

Results of this study are a step towards the fine mapping of NHR resistance genes. When fine mapping has yielded a small enough interval; the forward genetics approach can be completed by physical mapping and map-based cloning (Peters et al., 2003). The exact association between markers and gene can then be determined. This approach has proved to be a reliable method using NHR in practical breeding and although there are still some problems to overcome (such as linkage drag); this method can greatly contribute to resistance breeding worldwide (Johnston et al., 2013).

5. Conclusions

This section will be dedicated to answering the research question. Only a short answer is given, for full background readers are referred to the results and discussion section.

Research questions *Rphq11*

Q_1. Using more markers and the same homozygous recombinants, is it possible to fine map the resistance to *Phs*/*Phb_Isr* and *Pp* more precisely at the 121-128cM interval?

A_1. It was not possible to fine map the region. The seven markers developed in this study identified no recombinations in the targeted region.

Q_2. Do the extra replications of the phenotypic tests corroborate the association between the phenotype and the markers of the locus 121-128cM?

A_2. Yes, association was found for the rusts *Phs*, *Phb_Isr, Phb_Iran* and also for *Pp* with *Rnhq121cM*. This was proved with QTL analysis and a substitution mapping approach.

Q_3. Are any new associations found between markers and the heterologous rusts *Pgl*, *Phm* and *Pt*, using the (new) phenotypic and genotypic data of the homorecombinants?

A_3. Yes, association was found to the rusts *Phm* and *Pt* using the two methods described above. The association of *Pgl* with *Rnhq121cM* was only found by means of the QTL analysis.

Q_4. Is there an interaction between the different resistance loci for heterologous rusts *Phs*, *Pp*, *Pgl*, *Pt_S*, *Phm_R, Phb_Iran* and *Phb_Isr* and is there interaction between *Rphq11* and those resistance loci; assuming that the resistance QTLs can be mapped using the data from this study.

A_4. A two locus interaction was only observed for *Phb_Iran*, for the QTLS *Rphq11* and *Rnhq121cM*. A trend was found for *Pp*, *Pt* and *Phm*. No additional two-locus interactions were found.

Q_5. Using the new phenotypic data, are there any new regions found that confer resistance to the heterologous pathogens in SusQ11?

A_5. Only association with additional pathogens (*Pgl*, *Phm*, *Pt*), in comparison with Salunke (2013), was found for the *Rnhq121cM* region, no additional regions were found. A region of SusPtrit was associated with resistance; however this association was only just significant and only found in the QTL analysis.

Q_6. What is the histological effect of two locus interaction on *Phb_Iran*?

A_6. *Rnhq121cM* has a relatively large effect, in contrast with *Rphq11*, on the width and %EA of *Phb_Iran*, where *Rphq11* has an effect on the speed of the infection.

Research questions Resistance gene L94

Q_1. Using the markers from the study done by Chisenga (2013), is it possible to genotype the individuals in the progeny of 1501-2 to 1501-5? And can this information be used to identify individuals for (future) recombinant screenings?

A_1. Only markers 3 and 26 were useful in genotyping the progeny. Marker 7, 8, 13, 25 and 36 could not be genotyped using LightScanner and/or CAPs. As only two of the seven targeted SNPs were genotyped, recombinant screening was not possible.

Q_2. Are these markers associated with resistance to *Pp* and *Phb_Isr* in the progeny of 1501-2 to 1501-5?

A_2. Markers 3 and 26 are associated (either additive for the L94 allele or dominance for the SusPtrit allele) with resistance to *Pp*. On the other hand, they are not associated with resistance to *Phb_Isr*.

Q_3. Do these genotyped markers explain all of the phenotypic variation in the progeny of 1501-2 to 1501-5?

A_3. It is not possible to answer this question for *Phb_Isr*, because none of the two intersting markers were genotyped. For *Pp*, 2 of the 3 interesting markers were genotyped and they do not explain all the phenotypic variation observed.

6. References

Atienza, S.G., H. Jafary, and R.E. Niks, 2004: Accumulation of genes for susceptibility to rust fungi for which barley is nearly a nonhost results in two barley lines with extreme multiple susceptibility. Planta **220**, 71-79.

Bent, A.F., and D. Mackey, 2007: Elicitors, effectors, and R genes: the new paradigm and a lifetime supply of questions. Annu. Rev. Phytopathol. **45**, 399-436.

Chen, X., C.A. Hackett, R.E. Niks, P.E. Hedley, C. Booth, A. Druka, T.C. Marcel, A. Vels, M. Bayer, and I. Milne, 2010: An eQTL analysis of partial resistance to *Puccinia hordei* in barley. PLoS One **5**, e8598.

Chisenga, T., R., 2013: Mapping of genes for non-host resistance of barley to rust and powdery mildew, Wageningen University and Research Centre.

den Boer, E., N.W. Zhang, K. Pelgrom, R.G. Visser, R.E. Niks, and M.J. Jeuken, 2013: Fine mapping quantitative resistances to downy mildew in lettuce revealed multiple sub-QTLs with plant stage dependent effects reducing or even promoting the infection. Theoretical and Applied Genetics **126**, 2995-3007.

Devaux, P., A. Kilian, and A. Kleinhofs, 1995: Comparative mapping of the barley genome with male and female recombination-derived, doubled haploid populations. Molecular and General Genetics MGG **249**, 600-608.

Flor, H.H., 1971: Current status of the gene-for-gene concept. Annual review of phytopathology **9**, 275-296.

Hammond-Kosack, K.E., and J.E. Parker, 2003: Deciphering plant–pathogen communication: fresh perspectives for molecular resistance breeding. Current Opinion in Biotechnology **14**, 177-193.

Heath, M.C., 2000: Nonhost resistance and nonspecific plant defenses. Current opinion in plant biology **3**, 315-319.

Jafary, H., 2006: Barley-*Puccinia* rusts: a model system to study the genetics, evolution and mechanisms of nonhost immunity in plants Wageningen University.

Jafary, H., L.J. Szabo, and R.E. Niks, 2006a: Innate nonhost immunity in barley to different heterologous rust fungi is controlled by sets of resistance genes with different and overlapping specificities. Molecular plant-microbe interactions **19**, 1270-1279.

Jafary, H., G. Albertazi, and R. Niks, 2006b: Diversity of loci carrying genes for resistance of barley to heterologous rust species Proceedings of the non-specific and specific innate and acquired plant resistance symposium, Vol. 31.

Jafary, H., G. Albertazzi, T.C. Marcel, and R.E. Niks, 2008: High diversity of genes for nonhost resistance of barley to heterologous rust fungi. Genetics **178**, 2327-2339.

Jeuken, M.J., 2002: The genetics of non-host resistance to the lettuce pathogen Bremia lactucae in Lactuca saligna Wageningen Universiteit.

Johnston, P.A., R.E. Niks, V. Meiyalaghan, E. Blanchet, and R. Pickering, 2013: Rph22: mapping of a novel leaf rust resistance gene introgressed from the non-host *Hordeum bulbosum* L. into cultivated barley (*Hordeum vulgare* L.). Theoretical and Applied Genetics **126**, 1613-1625.

Jones, J.D., and J.L. Dangl, 2006: The plant immune system. Nature **444**, 323-329.

Kuijken, R., 2009: Characterization of Rphq11, a QTL for quantitative resistance to barley leaf rust., Wageningen University.

Lipka, U., R. Fuchs, and V. Lipka, 2008: Arabidopsis non-host resistance to powdery mildews. Current opinion in plant biology **11**, 404-411.

Lu, H., J. Romero-Severson, and R. Bernardo, 2002: Chromosomal regions associated with segregation distortion in maize. Theoretical and Applied Genetics **105**, 622-628.

Marcel, T.C., 2007: Genetic architecture of basal resistance of barley to *Puccinia hordei* Wageningen Universiteit.

Marcel, T.C., R. Aghnoum, J. Durand, R.K. Varshney, and R.E. Niks, 2007a: Dissection of the barley 2L1. 0 region carrying the '*Laevigatum*' quantitative resistance gene to leaf rust using near-isogenic lines (NIL) and subNIL. Molecular Plant-Microbe Interactions **20**, 1604-1615.

Marcel, T.C., R. Varshney, M. Barbieri, H. Jafary, M. De Kock, A. Graner, and R. Niks, 2007b: A high-density consensus map of barley to compare the distribution of QTLs for partial resistance to *Puccinia hordei* and of defence gene homologues. Theoretical and Applied Genetics **114**, 487-500.

McDonald, B.A., and C. Linde, 2002: Pathogen population genetics, evolutionary potential, and durable resistance. Annual review of phytopathology **40**, 349-379.

Niks, R., 1982: Early abortion of colonies of leaf rust, *Puccinia hordei*, in partially resistant barley seedlings. Canadian Journal of Botany **60**, 714-723.

Niks, R., 1983a: Comparative histology of partial resistance and the nonhost reaction to leaf rust pathogens in barley and wheat seedlings. Phytopathology **73**, 60-64.

Niks, R., 1983b: Haustorium formation by *Puccinia hordei* in leaves of hypersensitive, partially resistant, and nonhost plant genotypes. Phytopathology **73**, 64-66.

Niks, R., 1988: Nonhost plant species as donors for resistance to pathogens with narrow host range. II. Concepts and evidence on the genetic basis of nonhost resistance. Euphytica **37**, 89-99.

Niks, R.E., and T.C. Marcel, 2009: Nonhost and basal resistance: how to explain specificity? New Phytologist **182**, 817-828.

Niks, R.E., J. Parlevliet, P. Lindhout, and Y. Bai, 2011: Breeding crops with resistance to diseases and pests Wageningen Academic Publishers.

Nuernberger, T., and V. Lipka, 2005: Non-host resistance in plants: new insights into an old phenomenon. Molecular Plant Pathology **6**, 335-345.

Parlevliet, J., and J. Zadoks, 1977: The integrated concept of disease resistance: a new view including horizontal and vertical resistance in plants. Euphytica **26**, 5-21.

Perfect, S.E., and J.R. Green, 2001: Infection structures of biotrophic and hemibiotrophic fungal plant pathogens. Molecular Plant Pathology **2**, 101-108.

Peters, J.L., F. Cnudde, and T. Gerats, 2003: Forward genetics and map-based cloning approaches. Trends in plant science **8**, 484-491.

Qi, X., G. Jiang, W. Chen, R.E. Niks, P. Stam, and P. Lindhout, 1999: Isolate-specific QTLs for partial resistance to *Puccinia hordei* in barley. Theoretical and Applied Genetics **99**, 877-884.

Qi, X., F. Fufa, D. Sijtsma, R.E. Niks, P. Lindhout, and P. Stam, 2000: The evidence for abundance of QTLs for partial resistance to *Puccinia hordei* on the barley genome. Molecular Breeding **6**, 1-9.

Remington, D., M. Ungerer, and M. Purugganan, 2001: Map-based cloning of quantitative trait loci: progress and prospects. Genetical Research **78**, 213-218.

Salunke, A., 2013: Fine Mapping of Quantitative Trait Loci for Nonhost Resistance to Rusts in Barley, Wageningen University.

van Dijk, T., 2007: Characterization of a QTL for Non-Host Resistance in Barley, Wagingen.

Yeo, F.K.S., 2007: Genetic Dissection of QTLs for partial resistance of barley to *Puccinia hordei*, Wageningen University.

Yuan, J., M. Haroon, D.A. Lightfoot, Y. Pelletier, Q. Liu, and X.-Q. Li, 2009: A high-resolution melting approach for analyzing allelic expression dynamics.

7. Appendices

7.1 SusQ11

7.1.1 Genotyping

Table 21. Molecular markers and their respective forward and reverse primers which were used for fine mapping of the 121-128cM region. Primers were constructed during this study. The array locus name is given in the column "locus". CM indicates consensus map.

Name	Marker	Position on CM (cM)	Forward	Reverse
62	SCRI_RS_48964	121.05	GTTGTTCGCCCATTGAGTTT	ACTGAGAGGATGGCATTCGT
63	SCRI_RS_116575	121.05	GAAAGGAAGGGAAAGGAAGC	AAAGCTGAATCAGAGCATCG
64	BOPA1_6996-838	121.05	GTGGAGGAATGCTTGTCACC	ATAGCAGCACTCGACGACCT
65	SCRI_RS_223885	121.25	CCTCATCCTCCTCCTCCTCT	CTTCCGAACATAAAGCAGCA
66	BOPA1_111-499	121.48	TGCGATTTACGAGGAGAACA	AGATCCTACGACGAGGCAAA
67	BOPA2_12_31402	123.16	CGATGAAGTGCGTGAGACAC	TCGAAGCAAGCAAGATCACA
68	BOPA1_871-462	123.34	AAGGCTTCTTCGGGTTCG	AACGCAGGTGTTTTTCTTCC
69	SCRI_RS_139831	123.72	CCATCTTGTCGTCAGGGTTT	CCATTATCACAGGCACAACG
70	SCRI_RS_224624	123.72	ACCTTGGTGATGGGAATCTG	AGGCCATTAATTGCACTGCT
71	BOPA2_12_21396	124.51	TGCGGAGTTATGTACTATGAGCA	CTCCCATCCAACTCAGCAGT
72	SCRI_RS_222093	126.62	ACCAGTTGGCTGCTCTCATT	TCTATTTGCGGCTCTTCCTC
73	BOPA1_14832-296	126.62	GGTCCTCATCATCCAATCCA	CCGCTTTTGGTGAACTCTGA
74	BOPA1_3180-1771	126.62	GAATAAACTGGAGCGTGAGGAG	CACACATCCACACAGGCATT
75	SCRI_RS_219799	127.53	CGGCGTACACCGACTACCT	CAGTGGAGCTGCTTCTTTGG

Table 22. Molecular markers and their respective forward and reverse primers which were used for the construction of KASPar markers. The locus name of the array is given in the column "locus". CM indicates consensus map. The markers k04002 and WBE139 were not based on the 9K i-select Infinium array. K04002 and WBE129 are EST marker; ESTs were taken from a database http://compbio.dfci.harvard.edu/cgi-bin/tgi/gimain.pl?gudb=barley, the locus name indicates the database name for that EST sequence.

Name	Marker	Position on CM (cM)	Forward	Reverse	Source
7*	SCRI_RS_230508	120.65	AGTGCATCAGGTGGAGGAAG	GCTCAGCAGCTTATCGGAAG	Salunke (2013)
25*	SCRI_RS_179560	121.05	GAATTGTGCTCTGCCTCTCC	AACCACCCAAAACTGAATGC	Salunke (2013)
38*	SCRI_RS_142593	131.87	CAGTCATGGCAACTGGGAAC	GCAAAACTGCGAGTCCTCTT	Salunke (2013)
41*	SCRI_RS_149429	128.13	GTTCCGCAATGTCCTCTGAC	CCTTCTCCTCTCCCTCGATT	Salunke (2013)
47*	SCRI_RS_157929	139.45	CGAGAGGATGAAGGTCAAGG	TCCTGCCAACGAATCAAGTA	Salunke (2013)
53*	SCRI_RS_135248	105.72	TCCATCCACTCCGAAGTTCT	TGTTCCAAAAATCTCCTCTGC	Salunke (2013)
K04002*	TC134078	89.8	GACACAGGACCTGAAGCACA	CGGCAGGCTCTACTATGAGG	Hori et al. (2005)
WBE129*	TC131656	94.5	CCCCCAAACTCCCAACT	CTCCAGCCAGCAGGTCTAA	Yeo et al (unpublished).

7.1.2 Phenotyping

Table 23. Phenotypic results for the inoculation performed with pathogen *Phs* for each homorecombinant and parental line. The measured IF and the subsequently calculated RIF is given for each replicate. The first replicate is performed by Salunke (2013). The average RIF is displayed for completeness. Colors indicate highest and lowest value, from dark green (lowest) to dark red (highest).

Pathogen	Genotype	IF_1	IF_2	IF_3	RIF_1	RIF_2	RIF_3	Average RIF
Phs	SusPtrit	9.1	4.7	8.4	100	100.0	100.0	100.0
Phs	Steptoe	0	0.0	0.0	0	0.0	0.0	0.0
Phs	SusQ11	2.2	1.2	0.5	19.5	24.9	5.5	16.6
Phs	Rec 1	8.6	3.4	6.5	75.7	73.9	77.2	75.6
Phs	Rec 2	1.4	0.7	0.7	12.2	14.6	8.7	11.9
Phs	Rec 3	3.9	5.2	2.1	131.8	110.7	24.6	89.0
Phs	Rec 4	5.5	1.7	0.5	46.4	36.2	6.4	29.7
Phs	Rec 5	7.4	4.8	10.3	112.6	102.7	122.4	112.6
Phs	Rec 6	1.1	6.7	5.6	34.5	144.9	66.1	81.8
Phs	Rec 7	1.8	4.3	3.4	92.7	92.8	40.0	75.2
Phs	Rec 8	1.4	5.6	4.5	100.2	121.2	53.5	91.6
Phs	Rec 9	1.4	5.6	1.8	70.3	120.7	21.2	70.7
Phs	Rec 10	6.0	6.9	3.9	48.7	147.3	46.2	80.8
Phs	Rec 11	9.1	6.5	7.2	65.1	139.9	85.2	96.7
Phs	Rec 12	3.2	2.7	0.2	36.7	57.6	1.9	32.1
Phs	Rec 13	7.8	1.7	1.6	10.1	36.0	18.8	21.6
Phs	Rec 13'	6.4	0.9	0.2	15.9	20.4	1.8	12.7
Phs	Rec 14	6.9	1.1	2.3	30.9	23.9	27.5	27.4
Phs	Rec 15	4.9	1.5	0.4	12.6	33.3	4.7	16.9
Phs	Rec 16	2.5	3.3	0.9	18.8	70.4	10.6	33.3
Phs	Rec 17	2.1	2.0	0.7	28.6	42.6	8.6	26.6
Phs	Rec 18	1.3	2.4	1.0	12.3	52.2	11.5	25.3
Phs	Rec 19	2.0	2.8	0.4	18.1	60.0	4.8	27.6
Phs	Rec 20	1.2	8.9	8.3	53.1	190.5	98.4	114.0

Table 24. Phenotypic results for the inoculation performed with pathogen *Phb_Isr* for each homorecombinant and parental line. The measured IF and the subsequently calculated RIF is given for each replicate. The first replicate is performed by Salunke (2013). The average RIF is displayed for completeness. Colors indicate highest and lowest value, from dark green (lowest) to dark red (highest).

Pathogen	Genotype	IF_1	IF_2	IF_3	IF_4	RIF_1	RIF_2	RIF_3	RIF_4	Average RIF
Phb_Isr	SusPtrit	5.4	0.9	11.2	4.9	100	100.0	100.0	100	100.0
Phb_Isr	Steptoe	0	0.0	0.1	0.5	0	0.0	0.9	10.2	2.8
Phb_Isr	SusQ11	0.4	0.1	0.2	1.1	7.4	9.0	1.9	22.4	10.2
Phb_Isr	Rec 1	1.7	0.7	14.8	3.8	33.1	76.6	131.7	77.6	79.7
Phb_Isr	Rec 2	0.3	0.3	1.8		6.1	31.4	16.0		17.9
Phb_Isr	Rec 3	3.4	1.9	6.9		65.8	208.4	61.6		111.9
Phb_Isr	Rec 4	0.5	0.8	1.9	1.9	10.2	85.5	17.3	38.8	37.9
Phb_Isr	Rec 5	3.0	0.7	8.5		58.1	82.5	75.3		72.0
Phb_Isr	Rec 6	2.1	0.2	8.6	4.4	26.1	26.8	76.3	89.8	54.8
Phb_Isr	Rec 7	0.7	1.9	7.0		38.1	207.2	62.8		102.7
Phb_Isr	Rec 8	0.5	1.7	6.5		45.7	194.3	57.9		99.3
Phb_Isr	Rec 9	0.7	1.6	8.8		40.8	174.4	78.7		98.0
Phb_Isr	Rec 10	0.4	1.5	6.7		49.0	164.4	59.9		91.1
Phb_Isr	Rec 11	2.8	1.1	12.6		76.1	123.6	112.4		104.0
Phb_Isr	Rec 12	1.5	0.2	1.0		15.2	20.5	8.9		14.9
Phb_Isr	Rec 13	2.1	0.3	0.9		23.5	34.2	7.8		21.8
Phb_Isr	Rec 13'	2.6	0.4	0.5		14.2	41.4	4.8		20.1
Phb_Isr	Rec 14	2.7	0.3	0.8		19.3	38.4	7.4		21.7
Phb_Isr	Rec 15	4.3	0.3	0.5		10.0	32.3	4.3		15.6
Phb_Isr	Rec 16	0.9	0.6	0.8		14.1	62.9	7.1		28.0
Phb_Isr	Rec 17	1.3	0.2	2.0		17.7	26.8	18.0		20.8
Phb_Isr	Rec 18	1.1	1.2	1.6	1.2	9.6	129.8	14.5	24.5	44.6
Phb_Isr	Rec 19	1.0	0.5	1.0		8.2	54.2	8.6		23.7
Phb_Isr	Rec 20	0.5	1.5	13.3		54.4	168.1	118.8		113.7

Table 25. Phenotypic results for the inoculation performed with pathogen *Pt* for each homorecombinant and parental line. The measured IF and the subsequently calculated RIF is given for each replicate. The first replicate is performed by Salunke (2013). The average RIF is displayed for completeness. Colors indicate highest and lowest value, from dark green (lowest) to dark red (highest).

Pathogen	Genotype	IF_1	IF_2	IF_3	RIF_1	RIF_2	RIF_3	Average RIF
Pt	SusPtrit	21.1	24.7	17.2	100	100.0	100.0	100.0
Pt	Steptoe	0	0.0	0.1	0	0.1	0.8	0.3
Pt	SusQ11	9.1	5.5	5.7	43.1	22.3	33.2	32.9
Pt	Rec 1	5.0	19.8	17.5	53.2	79.9	102.2	78.4
Pt	Rec 2	5.3	6.9	7.7	31.0	27.9	44.9	34.6
Pt	Rec 3	14.6	17.6	15.6	89.8	71.2	90.7	83.9
Pt	Rec 4	4.3	8.5	5.6	33.2	34.3	32.5	33.3
Pt	Rec 5	9.6	21.5	18.1	90.6	86.9	105.4	94.3
Pt	Rec 6	10.7	28.2	17.8	26.6	114.2	103.5	81.4
Pt	Rec 7	10.8	21.1	17.6	59.6	85.5	102.5	82.5
Pt	Rec 8	6.4	20.0	15.0	76.9	81.0	87.1	81.7
Pt	Rec 9	4.4	27.6	19.5	66.2	111.6	113.4	97.1
Pt	Rec 10	2.3	31.0	16.8	49.4	125.5	97.9	90.9
Pt	Rec 11	13.9	31.8	24.9	69.6	128.8	145.0	114.5
Pt	Rec 12	23.4	7.7	5.8	30.6	31.1	33.8	31.9
Pt	Rec 13	20.1	8.8	4.4	44.9	35.7	25.5	35.4
Pt	Rec 13'	12.9	5.4	4.7	67.0	21.9	27.5	38.8
Pt	Rec 14	18.2	7.0	6.7	37.3	28.5	38.8	34.8
Pt	Rec 15	8.0	5.1	5.2	39.8	20.6	30.2	30.2
Pt	Rec 16	11.7	8.9	5.5	38.3	35.9	32.2	35.5
Pt	Rec 17	9.7	6.5	7.2	22.1	26.5	41.9	30.2
Pt	Rec 18	10.0	8.1	6.7	27.2	32.6	38.7	32.8
Pt	Rec 19	5.8	6.0	3.6	14.2	24.2	21.1	19.8
Pt	Rec 20	19.7	21.1	18.3	75.4	85.6	106.7	89.2

Table 26. Phenotypic results for the inoculation performed with pathogen *Pgl* for each homorecombinant and parental line. The measured IF and the subsequently calculated RIF is given for each replicate. The first replicate is performed by Salunke (2013). The average RIF is displayed for completeness. Colors indicate highest and lowest value, from dark green (lowest) to dark red (highest).

Pathogen	Genotype	IF_1	IF_2	IF_3	IF_4	IF_5	RIF_1	RIF_2	RIF_3	RIF_4	RIF_5	Average RIF
Pgl	SusPtrit	16.8	3.0	7.6	16.4	9.5	100.0	100.0	100.0	100.0	100	100.0
Pgl	Steptoe	3.3	0.1	0.6	0.9	0.1	19.7	3.0	7.4	5.4	1.1	7.3
Pgl	SusQ11	6.5	1.7	2.3	3.2	3.2	38.9	57.7	30.8	19.7	33.7	36.1
Pgl	Rec 1	13.5	2.7	7.1			117.3	89.4	93.2			100.0
Pgl	Rec 2	10.0	1.6	4.5	11.3		87.3	52.8	59.0	68.9		67.0
Pgl	Rec 3	17.3	3.6	16.0			150.5	120.7	211.0			160.7
Pgl	Rec 4	25.8	1.7	7.0	19.2		36.5	55.8	92.9	116.5		75.4
Pgl	Rec 5	15.5	5.6	15.2			224.8	187.0	200.9			204.2
Pgl	Rec 6	9.0	4.0	5.7			48.5	132.1	74.6			85.1
Pgl	Rec 7	6.4	4.9	9.5			135.1	163.1	125.5			141.2
Pgl	Rec 8	8.6	4.6	13.2			70.0	154.3	174.1			132.8
Pgl	Rec 9	8.7	3.7	10.6			78.4	123.0	140.1			113.8
Pgl	Rec 10	10.3	3.3	10.2			62.0	111.5	134.2			102.6
Pgl	Rec 11	6.8	3.9	9.5			83.7	131.2	125.8			113.6
Pgl	Rec 12	8.0	1.1	1.7			55.9	35.1	22.3			37.8
Pgl	Rec 13	10.7	0.9	4.5			24.7	31.3	59.6			38.5
Pgl	Rec 13'	15.4	1.8	3.0			74.8	61.4	40.2			58.8
Pgl	Rec 14	13.6	1.3	5.4			57.7	45.0	71.2			58.0
Pgl	Rec 15	18.4	1.0	4.0			28.1	32.4	52.3			37.6
Pgl	Rec 16	5.4	1.1	2.6			76.0	37.2	34.6			49.2
Pgl	Rec 17	12.7	2.0	3.8			39.1	68.0	50.3			52.5
Pgl	Rec 18	6.2	3.4	2.9		2.8	89.2	113.7	38.5		29	67.7
Pgl	Rec 19	8.6	2.0	5.5			58.7	68.1	72.8			66.6
Pgl	Rec 20	16.2	6.6	8.1	11.7		73.6	219.9	107.0	71.1		117.9

Table 27. Phenotypic results for the inoculation performed with pathogen *Phm* for each homorecombinant and parental line. The measured IF and the subsequently calculated RIF is given for each replicate. The first replicate is performed by Salunke (2013). The average RIF is displayed for completeness. Colors indicate highest and lowest value, from dark green (lowest) to dark red (highest).

Pathogen	Genotype	IF_1	IF_2	IF_3	RIF_1	RIF_2	RIF_3	Average RIF
Phm	SusPtrit	5.1	11.1	16.2	100.0	100.0	100.0	100.0
Phm	Steptoe	0	0.0	0.1	0.0	0.0	0.8	0.3
Phm	SusQ11	1.9	0.6	2.0	37.3	5.5	12.2	18.3
Phm	Rec 1	2.5	5.9	7.4	45.2	53.1	45.6	47.9
Phm	Rec 2	2.0	2.2	4.5	35.7	19.7	28.0	27.8
Phm	Rec 3	4.7	8.1	13.7	84.2	72.8	84.3	80.4
Phm	Rec 4	1.1	1.4	3.7	19.8	12.3	23.1	18.4
Phm	Rec 5	2.5	13.4	9.5	44.7	121.2	58.9	74.9
Phm	Rec 6	2.3	8.6	12.1	41.8	77.0	74.4	64.4
Phm	Rec 7	4.2	10.7	7.0	74.7	96.1	43.0	71.3
Phm	Rec 8	3.2	10.1	12.8	56.6	91.0	78.9	75.5
Phm	Rec 9	3.1	11.4	13.8	55.7	102.7	85.1	81.1
Phm	Rec 10	2.9	10.8	12.4	52.3	97.4	76.4	75.3
Phm	Rec 11	1.3	10.7	9.8	102.6	96.8	60.4	86.6
Phm	Rec 12	4.6	1.6	4.3	55.0	14.2	26.7	32.0
Phm	Rec 13	2.5	1.4	3.9	37.8	12.7	23.8	24.8
Phm	Rec 13'	1.7	1.0	3.8	23.5	9.4	23.3	18.7
Phm	Rec 14	2.9	1.6	3.4	65.0	14.0	21.1	33.4
Phm	Rec 15	1.7	1.7	3.7	38.7	14.9	23.1	25.6
Phm	Rec 16	2.8	2.1	5.1	62.5	18.9	31.2	37.5
Phm	Rec 17	2.0	2.5	4.3	44.4	22.6	26.7	31.2
Phm	Rec 18	2.4	3.4	4.2	54.3	30.8	25.8	37.0
Phm	Rec 19	1.6	2.8	3.3	35.6	25.5	20.3	27.1
Phm	Rec 20	3.7	5.7	17.1	82.0	51.0	105.6	79.5

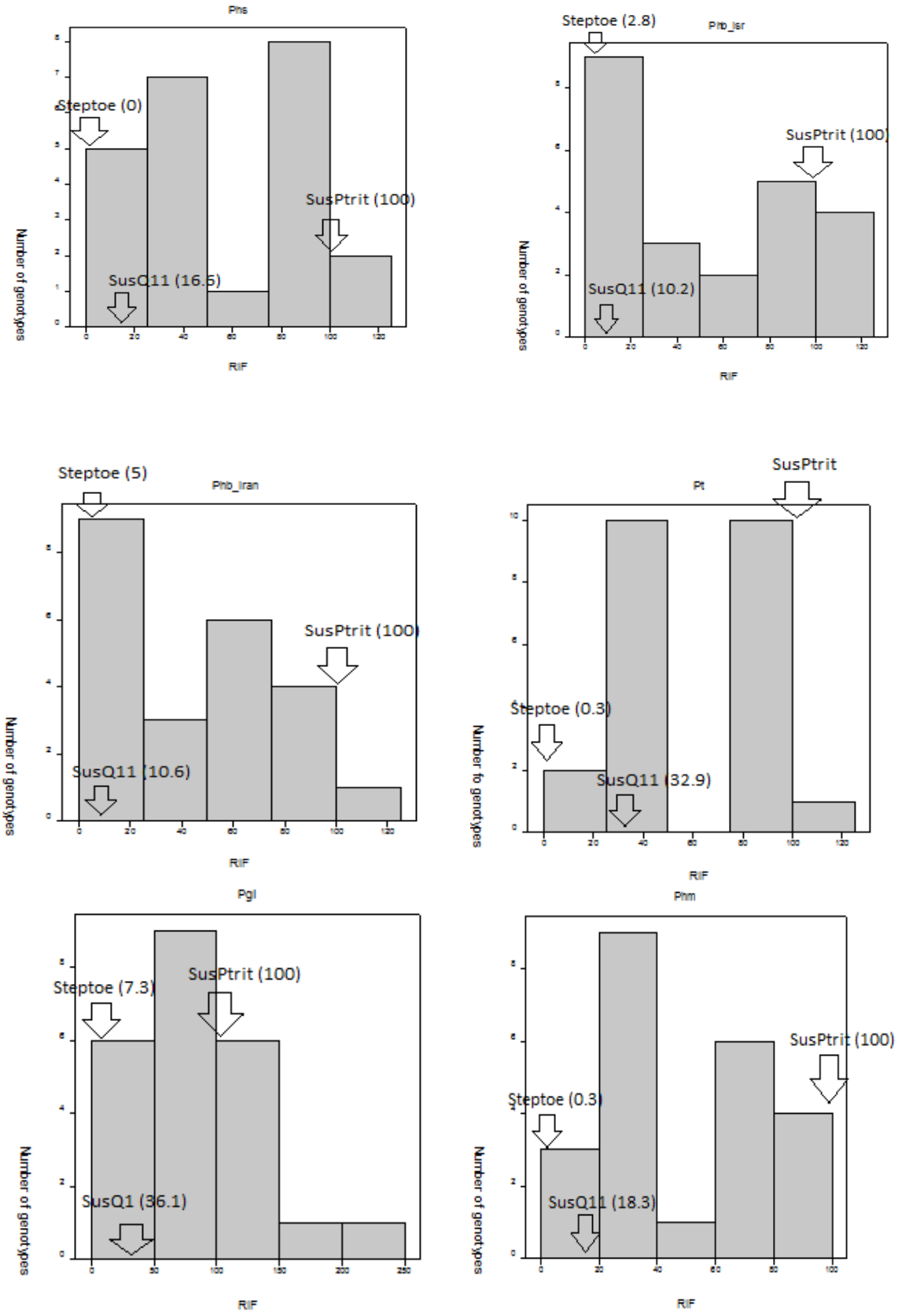


Figure 20. Frequency distribution of the average RIF for the pathogens (from left to right) *Phs*, *Phb_Isr*, *Phb_Iran*, *Pt*, *Pgl* and *Phm*. The RIF of the parental lines is indicated by an arrow, the exact value is shown between parentheses. Y-axis: number of genotypes; x-axis: RIF.

7.1.3 QTL analysis

Table 28. Markers used in the QTL mapping analysis. Number in the table(s), marker name, array locus name (if based on array sequence) and position on chromosome 2H in cM is given. An asterisk indicates that the exact position of the marker is uncertain.

Number	Marker	Locus (array)	Position (cM)
1	WBE129		89.8
2	TC161220-1		90*
3	WBE144 (***Rphq11***)		91.28
4	TC168528		100.26
5	TC174372		100.26
6	GBM1062		100.26
7	53	SCRI_RS_135248	105.7
8	57	BOPA2_12_30555	110.3
9	56	BOPA2_12_30555-2	110.3
10	61	BOPA1_ConsensusGBS0348-2	112.33
11	1	SCRI_RS_147203	119.71
11	8	SCRI_RS_230508	120.65
12	25	SCRI_RS_179560	121.046
12	62	SCRI_RS_48964	121.046
12	64	BOPA1_6996-838	121.046
12	68	BOPA1_871-462	123.338
12	69	SCRI_RS_139831	123.72
12	73	BOPA1_14832-296	126.62
12	74	BOPA1_3180-1771	126.62
12	75	SCRI_RS_219799	127.53
13	41	SCRI_RS_149429	128.13
14	38	SCRI_RS_142593	131.87
15	45	SCRI_RS_192711	134.23
15	35	SCRI_RS_151129	135.02
16	47	SCRI_RS_157929	139.45
16	49	BOPA2_12_10579	144.62
16	21	SCRI_RS_118062	145.742
17	23	SCRI_RS_193100	146.48

Table 29. Results of the QTL analysis, per marker and per replicate, for *Pp*. Markers are numbered as indicated in table 16, in increasing order along chromosome 2H (cM). For each allele (A or B) the average RIF is given, per replicate. The total marker average per allele is also given (Avg. A/Avg. B). The ANOVA p-value is given for the factor allele and the interaction of allele and replicate. The p-value of replicate is not given. Significant p-values (α=0.001) are given in bold. Allele "A" = Steptoe, Allele "B" = SusPtrit. An asterisk indicates that the exact position of the marker is uncertain. This analysis was performed before blasting of marker WBE129 to the barley genome.

Pp	Numb.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	cM/allele	90*	90*	91.28	100.26	100.26	100.26	105.7	110.3	110.3	112.33	119.71	127.53	128.13	131.87	134.23	139.45	146.48
Repl.1	*A*	86.06	84.28	73.84	72.64	50.19	46.67	39.66	27.09	43.47	61.91	41.64	26.27	31.73	34.19	33.26	34.04	35.11
Repl.1	*B*	63.81	63.81	78.26	78.94	108.13	106.7	108.13	128.06	116.87	94.78	127.87	128.95	132.12	139.45	130.14	120.47	106.36
Repl.2	*A*	49.35	49.98	45.65	39.23	36.1	36.27	32.32	32.86	37.57	39.47	35.44	26.8	30.22	35.45	36.96	38.09	35.44
Repl.2	*B*	49.32	49.32	57.26	63.28	67.35	64.33	65.61	68.05	65.44	65.58	71.86	74.66	74.99	71.83	66.23	62.34	60.65
Repl.3	*A*	48.89	53.49	47.31	42.01	36.59	26.08	22.57	25.93	30.26	34.29	29.42	23.74	27.94	32.03	28.54	29.55	23.73
Repl.3	*B*	39.72	39.73	47.89	54.67	61.7	70.89	70.37	71.06	69.93	68.07	75.66	73.45	72.96	71.59	72.18	67.1	65.8
Avg.A		61.43	62.58	55.6	51.29	40.96	36.34	31.51	28.63	37.1	45.56	35.5	25.6	29.96	33.89	32.92	33.9	31.43
Avg.B		50.95	50.95	61.13	65.63	79.06	80.64	81.37	89.05	84.08	76.14	91.79	92.35	93.36	94.29	89.52	83.31	77.6
Repl. x allele		0.873	0.848	0.713	0.877	0.575	0.61	0.53	0.051	0.335	0.974	0.237	0.082	0.085	0.034	0.06	0.108	0.349
Allele effect		0.668	0.42	0.955	0.319	0.006	**0.001**	**2.27E-04**	**5.69E-04**	**5.69E-04**	0.034	**3.11E-05**	**1.11E-07**	**7.87E-07**	**3.54E-06**	**1.38E-05**	**1.89E-04**	**7.26E-04**

Table 30. Results of the QTL analysis, per marker and per replicate, for *Phs*. Markers are numbered as indicated in table 16, in increasing order along chromosome 2H (cM). For each allele (A or B) the average RIF is given, per replicate. The total marker average per allele is also given (Avg. A/Avg. B). The ANOVA p-value is given for the factor allele and the interaction of allele and replicate. The p-value of replicate is not given. Significant p-values (α=0.001) are given in bold. Allele "A" = Steptoe, Allele "B" = SusPtrit. An asterisk indicates that the exact position of the marker is uncertain. This analysis was performed before blasting of marker WBE129 to the barley genome.

Phs	Numb.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	cM/allele	90*	90*	91.28	100.26	100.26	100.26	105.7	110.3	110.3	112.33	119.71	127.53	128.13	131.87	134.23	139.45	146.48
Repl.1	*A*	57.6	61.79	55.64	49.37	48.92	40.77	33.02	32.26	35.19	35.13	33.54	28.847	33.76	38.5	37.89	38.66	38.48
Repl.1	*B*	32.8	32.76	37.02	48.9	49.49	58.33	63.96	67.61	67.34	70.99	73.47	71.33	69.2	65.75	63.82	60.63	57.38
Repl.2	*A*	69.7	72.26	69.54	63.78	55.91	53.34	52.25	60.63	65.24	70.94	61.63	45.97	49.57	54.69	56.11	57.23	52.35
Repl.2	*B*	85.7	85.69	94.15	96.72	106.95	105.11	101.8	97.16	94.81	89.25	103.72	113.15	115.19	114.52	106.69	100.86	97.91
Repl.3	*A*	35.85	42.51	37.86	36.5	32.08	24.98	24.66	24.9	24.62	27.58	23.48	17.43	19.16	21.62	22.79	23.97	21.39
Repl.3	*B*	29.13	29.13	34.49	36.94	42.68	49.47	47.72	49.55	52.39	50.87	57.24	57.7	59.47	60.14	54.76	50.57	48.46
Avg.A		54.38	58.85	54.35	49.88	45.64	39.69	36.64	39.26	41.68	44.55	39.55	30.75	34.16	38.27	38.93	39.95	37.41
Avg.B		49.19	49.19	55.22	60.85	66.37	70.97	71.16	71.44	71.51	70.37	78.14	80.73	81.29	80.14	75.09	70.69	67.92
Repl. x allele		0.426	0.236	0.254	0.322	0.038	0.303	0.499	0.856	0.983	0.767	0.933	0.334	0.305	0.339	0.538	0.627	0.518
Allele effect		0.045	0.347	0.935	0.287	0.092	0.002	**2.27E-04**	**0.001**	0.003	0.013	**1.34E-04**	**6.47E-08**	**6.215E-07**	**2.13E-05**	**2.70E-04**	0.002	0.002

Table 31. Results of the QTL analysis, per marker and per replicate, for *Phb_Isr*. Markers are numbered as indicated in table 16, in increasing order along chromosome 2H (cM). For each allele (A or B) the average RIF is given, per replicate. The total marker average per allele is also given (Avg. A/Avg. B). The ANOVA p-value is given for the factor allele and the interaction of allele and replicate. The p-value of replicate is not given. Significant p-values (α=0.001) are given in bold. Allele "A" = Steptoe, Allele "B" = SusPtrit. An asterisk indicates that the exact position of the marker is uncertain. This analysis was performed before blasting of marker WBE129 to the barley genome.

Phb_ISR	Numb.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	cM/allele	90*	90*	91.28	100.26	100.26	100.26	105.7	110.3	110.3	112.33	119.71	127.53	128.13	131.87	134.23	139.45	146.48
Repl.1	*A*	32.55	34.51	31.52	29.92	29.27	26.82	23.46	24.13	25.41	25.47	24.04	20.11	21.49	23.22	24.22	24.77	27.11
Repl.1	*B*	27.99	27.99	31.97	33.96	34.8	36.97	39.21	39.9	39.81	41.33	43.55	44.29	44.91	44.82	41.36	39.21	35.19
Repl.2	*A*	86.02	85.75	80.66	62.19	62.19	64.19	53.16	63.47	72	68.77	69.1	52.08	64.01	73.32	72.38	76.18	66.69
Repl.2	*B*	93.65	93.65	105.2	124.27	124.27	116.44	122.2	117.23	111.52	120.92	120.42	129.65	121.9	113.86	111.02	103.36	106.48
Repl.3	*A*	45.8	48.07	42.42	39.66	35.18	31.6	28.2	26.22	30.25	33.54	29.23	17.65	21.12	23.75	24.25	24.77	22.95
Repl.3	*B*	42.32	42.32	51.47	53.25	59.08	60.81	61.49	66.68	65.48	64.27	70.98	76.02	77.35	79.5	73.28	68.26	62.97
Avg.A		54.79	56.11	51.53	43.92	42.21	40.87	34.94	37.94	42.56	42.59	40.79	29.95	35.54	40.1	40.29	41.91	38.91
Avg.B		54.65	54.65	62.86	70.49	72.72	71.41	74.3	74.6	72.27	75.51	78.32	83.3	81.38	79.4	75.22	70.28	68.21
Repl. x allele		0.971	0.861	0.73	0.085	0.115	0.314	0.121	0.362	0.635	0.435	0.501	0.079	0.325	0.461	0.498	0.584	0.426
Allele effect		0.848	0.905	0.368	0.021	0.008	0.008	**4.45E-04**	**0.001**	0.012	0.006	**0.001**	**5.88E-07**	**4.73E-05**	**8.01E-04**	0.003	0.016	0.013

Table 32. Results of the QTL analysis, per marker and per replicate, for *Pt.* Markers are numbered as indicated in table 16, in increasing order along chromosome 2H (cM). For each allele (A or B) the average RIF is given, per replicate. The total marker average per allele is also given (Avg. A/Avg. B). The ANOVA p-value is given for the factor allele and the interaction of allele and replicate. The p-value of replicate is not given. Significant p-values (α=0.001) are given in bold. Allele "A" = Steptoe, Allele "B" = SusPtrit. An asterisk indicates that the exact position of the marker is uncertain. This analysis was performed before blasting of marker WBE129 to the barley genome.

Pt_Swiss	Numb.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	cM/allele	90*	90*	91.28	100.26	100.26	100.26	105.7	110.3	110.3	112.33	119.71	127.53	128.13	131.87	134.23	139.45	146.48
Repl.1	*A*	52.18	55.15	52.99	50.64	50.3	46.44	40.97	42.17	44.02	42.78	43.04	38.48	40.1	42.73	43.46	45.24	48.2
Repl.1	*B*	40.22	40.22	40.51	46.05	46.5	51.053	55.68	55.71	54.66	57.78	57.29	59.74	59.76	57.86	55.39	52.36	48.99
Repl.2	*A*	59.3	61.42	57	52.96	46.22	42.68	39.76	39.77	45.3	50.22	44.05	34.97	38.86	41.86	42.44	43.78	40.58
Repl.2	*B*	59.14	59.14	66.85	70.13	78.9	79.79	79.38	82.96	80.1	76.31	85.9	88.21	88.47	89.3	83.81	78.6	75.7
Repl.3	*A*	66.23	69.24	64.17	59.45	54.72	51.016	45.25	44.56	49.85	53.68	49.5	39.41	44.26	47.32	48.47	49.02	46.81
Repl.3	*B*	62.8	62.8	70.7	75.53	81.68	83.3	85.87	90.31	88	86.28	92.8	95.93	95.27	57.86	89.8	85.45	81.54
Avg.A		59.4	59.4	58.05	54.35	50.41	46.71	41.99	42.168	46.39	48.89	45.55	37.62	41.07	43.97	44.79	46.01	45.2
Avg.B		54.05	54.05	59.35	63.9	69.03	71.37	73.64	76.33	74.25	73.46	78.66	81.29	81.17	81.11	76.33	72.14	68.74
Repl. x allele		0.981	0.836	0.562	0.517	0.163	0.211	0.302	0.152	0.313	0.689	0.235	0.059	0.129	0.119	0.206	0.247	0.146
Allele effect		0.191	0.372	0.887	0.275	0.028	0.003	**1.10E-04**	**8.72E-04**	**8.72E-04**	0.005	**7.001E-05**	**7.20E-09**	**3.355E-07**	**4.70E-06**	**1.24E-04**	0.002	0.005

Table 33. Results of the QTL analysis, per marker and per replicate, for *Phm.* Markers are numbered as indicated in table 16, in increasing order along chromosome 2H (cM). For each allele (A or B) the average RIF is given, per replicate. The total marker average per allele is also given (Avg. A/Avg. B). The ANOVA p-value is given for the factor allele and the interaction of allele and replicate. The p-value of replicate is not given. Significant p-values (α=0.001) are given in bold. Allele "A" = Steptoe, Allele "B" = SusPtrit. An asterisk indicates that the exact position of the marker is uncertain. This analysis was performed before blasting of marker WBE129 to the barley genome.

Phm	Numb.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	cM/allele	90*	90*	91.28	100.26	100.26	100.26	105.7	110.3	110.3	112.33	119.71	127.53	128.13	131.87	134.23	139.45	146.48
Repl.1	*A*	54.41	53.66	53.25	51.35	51.55	54.99	52.34	52.7	52.93	52.13	51.7	48.84	50.83	51.24	53.66	54.43	57.04
Repl.1	*B*	52.8	52.8	53.36	55.81	55.55	51.42	54.16	53.94	53.76	55.1	55.76	58.14	56.48	56.48	52.8	52.05	50.4
Repl.2	*A*	47.37	53.04	48.23	41.25	37.07	31.6	28.38	26.86	32.69	35.87	31.18	24.26	29.79	34.16	35.84	36.94	30.36
Repl.2	*B*	46.06	46.06	53.35	61.39	66.83	70.09	68.83	75.25	72.51	72	78.37	78.1	76.29	74.66	68.42	64.26	65.12
Repl.3	*A*	46.78	47.7	44.97	42.5	38.83	37.38	32.42	36.64	40.37	42.8	39.48	30.18	31.17	34.58	35.46	36.19	37.63
Repl.3	*B*	45.53	46.53	50.13	52.3	57.08	56.99	59.91	57.8	55.07	52.92	58.08	64.86	67.04	65.72	61.46	58.29	53.78
Avg.A		49.52	49.52	48.81	45.04	42.48	41.33	37.71	38.73	41.99	43.6	40.98	34.43	37.26	39.99	41.65	42.52	41.68
Avg.B		48.13	48.13	52.3	56.5	59.82	59.5	61.3	62.33	60.45	60	64.08	67.03	66.61	65.62	60.89	58.2	56.44
Repl. x allele		0.49	0.947	0.677	0.706	0.382	0.067	0.073	0.025	0.097	0.181	0.057	0,020	0.042	0.123	0.153	0.23	0.083
Allele effect		0.391	0.675	0.96	0.146	0.025	0.015	**0.001**	**0.001**	0.014	0.035	0.002	**2.85E-06**	**4.58E-05**	**6.76E-04**	0.011	0.04	0.052

Table 34. Results of the QTL analysis, per marker and per replicate, for *Pgl.* Markers are numbered as indicated in table 16, in increasing order along chromosome 2H (cM). For each allele (A or B) the average RIF is given, per replicate. The total marker average per allele is also given (Avg. A/Avg. B). The ANOVA p-value is given for the factor allele and the interaction of allele and replicate. The p-value of replicate is not given. Significant p-values (α=0.001) are given in bold. Allele "A" = Steptoe, Allele "B" = SusPtrit. An asterisk indicates that the exact position of the marker is uncertain. This analysis was performed before blasting of marker WBE129 to the barley genome.

Pgl	Numb.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	cM/allele	90*	90*	91.28	100.26	100.26	100.26	105.7	110.3	110.3	112.33	119.71	127.53	128.13	131.87	134.23	139.45	146.48
Repl.1	*A*	76.9	88.25	83.42	80.48	79.76	72.07	62.34	56.55	58.23	57.53	60.31	57.69	63.64	64.1	66.22	68.48	70.84
Repl.1	*B*	60.9	60.86	63.07	70.96	71.9	81	89.18	97.94	99.9	105.6	101.29	96.69	92.85	95.39	89.5	84.92	80.57
Repl.2	*A*	86.4	94.17	86.25	75.1	70.17	65.25	61.61	72	75.92	79.94	74.27	58.07	66.15	72.45	73.73	74.21	62.57
Repl.2	*B*	90.8	90.79	104.8	115.58	122	122.65	121.2	115.28	114.51	112.55	121.37	130.48	127.21	124.21	117.37	112.88	115.88
Repl.3	*A*	92.8	101.2	93.45	84.79	82.92	68.56	56.86	61.01	67.1	67.63	66.52	57.02	62.28	70.27	68.53	70.05	67.92
Repl.3	*B*	76.3	76.3	84.52	97.57	100	114.11	121.03	122.34	120.57	125.67	127.4	126.7	126.82	121.57	118.7	112.49	107.6
Avg.A		85.38	94.52	87.71	80.12	77.61	68.63	60.27	63.19	67.08	68.37	67.03	57.59	64.03	68.94	69.49	70.91	67.11
Avg.B		75.99	75.99	84.13	94.7	97.97	105.92	110.47	111.85	111.66	114.61	116.69	117.96	115.63	113.72	108.52	103.43	101.35
Repl. x allele		0.845	0.706	0.487	0.285	0.157	0.231	0.341	0.737	0.864	0.684	0.779	0.356	0.375	0.732	0.642	0.65	0.336
Allele effect		**2.01E-04**	0.155	0.792	0.258	0.109	0.003	**3.342E-05**	**3.82E-04**	**3.82E-04**	**2.64E-04**	**7.978E-05**	**2.51E-07**	**2.25E-05**	**4.36E-04**	0.002	0.01	0.007

Table 35. Results of the QTL analysis, per marker, for *Phb_Iran*. Markers are numbered as indicated in table 16, in increasing order along chromosome 2H (cM). For each allele (A or B) the average RIF is given (Avg. A/Avg. B). The ANOVA p-value is given for the factor allele and the interaction of allele and replicate. The p-value of replicate is not given. Allele "A" = Steptoe, Allele "B" = SusPtrit. An asterisk indicates that the exact position of the marker is uncertain. This analysis was performed before blasting of marker WBE129 to the barley genome.

Phb_Iran	**Numb.**	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	1.60E+01	17
	cM/allele	90*	90*	91.28	100.26	100.26	100.26	105.7	110.3	110.3	112.33	119.71	127.53	128.13	131.87	134.23	139.45	146.48
Avg. A		28.21	30.86	27.13	21.63	19.8	17.18	16.87	19.77	25.83	28.25	24.16	11.713	1.61E+01	19.027	19.42	18.15	11.01
Avg. B		60.34	60.34	74.69	72.34	74.71	72.59	68.25	69.75	66.88	67.82	74.03	78.545	79.508	82.019	75.21	71.52	68.8
Allele effect		0.127	0.065	0.002	**4.47E-04**	**8.37E-05**	**5.49E-05**	**3.03E-04**	**3.03E-04**	0.007	0.011	**7.79E-04**	**1.07E-08**	**5.38E-07**	**1.54E-06**	**5.61E-05**	**1.37E-04**	**2.07E-05**

7.1.4 Substitution mapping

Table 36. SPSS output for the first part of the substitution mapping procedure of *Pp*. This table consists of 3 parts, namely an ANOVA table showing the significance values for the main effects "Genotype" and "Replicate" and the interaction, for the y-variate the logarithm of the RIF. The second part consists of the Dunnetts post-hoc test indicating whether SusPtrit and any other genotype are significant different. The third part contains the same Dunnett post-hoc test, the difference being that as a control group SusQ11 is taken. The genotypes are denoted by codes: 0= SusQ11, 1000=SusPtrit, 21=Steptoe, 1=homorecombinant 1, 2= homorecombinant 2 etc. α=0.05.

Tests of Between-Subjects Effects

Dependent Variable: logRIF

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	63.403[a]	76	.834	17.267	.000
Intercept	332.391	1	332.391	6879.644	.000
Genotype	44.239	23	1.923	39.810	.000
Replicate	1.225	3	.408	8.449	.000
Genotype * Replicate	7.130	50	.143	2.952	.000
Error	6.474	134	.048		
Total	551.868	211			
Corrected Total	69.877	210			

a. R Squared = .907 (Adjusted R Squared = .855)

Multiple Comparisons

Dependent Variable: logRIF

Dunnett t (2-sided)

(I) !Genotype	(J) !Genotype	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	1000	-1.027238*	.0872543	.000	-1.293537	-.760938
1	1000	-.052109	.0962311	1.000	-.345805	.241587
2	1000	-.788434*	.0962311	.000	-1.082130	-.494738
3	1000	.019153	.0962311	1.000	-.274543	.312850
4	1000	-.269039*	.0832921	.030	-.523246	-.014832
5	1000	-.087984	.0962311	.999	-.381680	.205712
6	1000	.130053	.0962311	.950	-.163643	.423750
7	1000	-.015734	.0962311	1.000	-.309430	.277962
8	1000	-.050922	.0962311	1.000	-.344618	.242775
9	1000	.109325	.1006141	.995	-.197748	.416398
10	1000	.108228	.0962311	.992	-.185468	.401925
11	1000	.004991	.0962311	1.000	-.288705	.298687
12	1000	-.911409*	.0962311	.000	-1.205105	-.617713
13	1000	-.731818*	.1006141	.000	-1.038891	-.424745
14	1000	-.760734*	.0962311	.000	-1.054430	-.467038
15	1000	-.969834*	.0962311	.000	-1.263530	-.676138
16	1000	-1.040222*	.0962311	.000	-1.333918	-.746525
17	1000	-.694947*	.0962311	.000	-.988643	-.401250
18	1000	-.661672*	.0962311	.000	-.955368	-.367975

19	1000	-.848659*	.0962311	.000	-1.142355	-.554963
20	1000	-.294797*	.0851310	.014	-.554616	-.034978
21	1000	-1.820683*	.0872543	.000	-2.086982	-1.554384
130	1000	-.683747*	.1006141	.000	-.990820	-.376674

Based on observed means.

The error term is Mean Square(Error) = .048.

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Multiple Comparisons

Dependent Variable: logRIF

Dunnett t (2-sided)

(I) !Genotype	(J) !Genotype	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	0	.975128*	.1021357	.000	.666716	1.283541
2	0	.238803	.1021357	.255	-.069609	.547216
3	0	1.046391*	.1021357	.000	.737979	1.354803
4	0	.758199*	.0900491	.000	.486283	1.030114
5	0	.939253*	.1021357	.000	.630841	1.247666
6	0	1.157291*	.1021357	.000	.848879	1.465703
7	0	1.011503*	.1021357	.000	.703091	1.319916
8	0	.976316*	.1021357	.000	.667904	1.284728
9	0	1.136562*	.1062754	.000	.815650	1.457475
10	0	1.135466*	.1021357	.000	.827054	1.443878
11	0	1.032228*	.1021357	.000	.723816	1.340641
12	0	.115828	.1021357	.985	-.192584	.424241
13	0	.295419	.1062754	.094	-.025493	.616332
14	0	.266503	.1021357	.142	-.041909	.574916
15	0	.057403	.1021357	1.000	-.251009	.365816
16	0	-.012984	.1021357	1.000	-.321396	.295428
17	0	.332291*	.1021357	.026	.023879	.640703
18	0	.365566*	.1021357	.009	.057154	.673978
19	0	.178578	.1021357	.667	-.129834	.486991
20	0	.732441*	.0917527	.000	.455381	1.009500
21	0	-.793445*	.0937261	.000	-1.076464	-.510427
130	0	.343491*	.1062754	.027	.022578	.664404
1000	0	1.027238*	.0872543	.000	.763762	1.290713

Based on observed means.

The error term is Mean Square(Error) = .048.

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table 37. SPSS output for the first part of the substitution mapping procedure of *Phs*. This table consists of 3 parts, namely an ANOVA table showing the significance values for the main effects "Genotype" and "Replicate" and the interaction, for the y-variate the logarithm of the RIF. The second part consists of the Dunnetts post-hoc test indicating whether SusPtrit and any other genotype are significant different. The third part contains the same Dunnett post-hoc test, the difference being that as a control group SusQ11 is taken. The genotypes are denoted by codes: 1=homorecombinant 1, 2= homorecombinant 2 etc. α=0.05.

Tests of Between-Subjects Effects

Dependent Variable: logRIF

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	50.742[a]	71	.715	17.130	.000
Intercept	375.504	1	375.504	9000.561	.000
Replicate	6.012	2	3.006	72.055	.000
Genotype	36.754	23	1.598	38.303	.000
Replicate * Genotype	5.166	46	.112	2.692	.000
Error	5.090	122	.042		
Total	479.055	194			
Corrected Total	55.831	193			

a. R Squared = .909 (Adjusted R Squared = .856)

Multiple Comparisons

Dependent Variable: logRIF

Dunnett t (2-sided)

(I) !Genotype	(J) !Genotype	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
SusQ	SusP	-.737934*	.0918057	.000	-1.013999	-.461870
1	SusP	-.076339	.0992500	1.000	-.374789	.222111
2	SusP	-.927539*	.0992500	.000	-1.225989	-.629089
3	SusP	-.150864	.0992500	.813	-.449314	.147586
4	SusP	-.635976*	.0992500	.000	-.934426	-.337527
5	SusP	-.024364	.0992500	1.000	-.322814	.274086
6	SusP	-.164160	.1029348	.760	-.473690	.145370
7	SusP	-.188864	.0992500	.519	-.487314	.109586
8	SusP	-.113539	.0992500	.978	-.411989	.184911
9	SusP	-.282551	.0992500	.076	-.581001	.015898
10	SusP	-.168939	.0992500	.677	-.467389	.129511
11	SusP	-.046389	.0992500	1.000	-.344839	.252061
12	SusP	-.760289*	.0992500	.000	-1.058739	-.461839
13	SusP	-.747332*	.1029348	.000	-1.056862	-.437802
14	SusP	-.687564*	.0992500	.000	-.986014	-.389114
15	SusP	-.886789*	.0992500	.000	-1.185239	-.588339
16	SusP	-.726214*	.0992500	.000	-1.024664	-.427764
17	SusP	-.675789*	.0992500	.000	-.974239	-.377339
18	SusP	-.697726*	.0992500	.000	-.996176	-.399277
19	SusP	-.851751*	.0992500	.000	-1.150201	-.553302

20	SusP	-.064464	.0992500	1.000	-.362914	.233986
Steptoe	SusP	-2.000389*	.0992500	.000	-2.298839	-1.701939
13'	SusP	-.913339*	.0992500	.000	-1.211789	-.614889

Based on observed means.

The error term is Mean Square(Error) = .042.

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Multiple Comparisons

Dependent Variable: logRIF

Dunnett t (2-sided)

(I) !Genotype	(J) !Genotype	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	SusQ	.661595*	.0949091	.000	.373800	.949391
2	SusQ	-.189605	.0949091	.483	-.477400	.098191
3	SusQ	.587070*	.0949091	.000	.299275	.874866
4	SusQ	.101958	.0949091	.992	-.185838	.389754
5	SusQ	.713570*	.0949091	.000	.425775	1.001366
6	SusQ	.573774*	.0987559	.000	.274313	.873235
7	SusQ	.549070*	.0949091	.000	.261275	.836866
8	SusQ	.624395*	.0949091	.000	.336600	.912191
9	SusQ	.455383*	.0949091	.000	.167587	.743179
10	SusQ	.568995*	.0949091	.000	.281200	.856791
11	SusQ	.691545*	.0949091	.000	.403750	.979341
12	SusQ	-.022355	.0949091	1.000	-.310150	.265441
13	SusQ	-.009397	.0987559	1.000	-.308858	.290063
14	SusQ	.050370	.0949091	1.000	-.237425	.338166
15	SusQ	-.148855	.0949091	.813	-.436650	.138941
16	SusQ	.011720	.0949091	1.000	-.276075	.299516
17	SusQ	.062145	.0949091	1.000	-.225650	.349941
18	SusQ	.040208	.0949091	1.000	-.247588	.328004
19	SusQ	-.113817	.0949091	.976	-.401613	.173979
20	SusQ	.673470*	.0949091	.000	.385675	.961266
Steptoe	SusQ	-1.262455*	.0949091	.000	-1.550250	-.974659
13'	SusQ	-.175405	.0949091	.599	-.463200	.112391
SusP	SusQ	.737934*	.0918057	.000	.459549	1.016320

Based on observed means.

The error term is Mean Square(Error) = .042.

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table 38. SPSS output for the first part of the substitution mapping procedure of *Phb_Isr*. This table consists of 3 parts, namely an ANOVA table showing the significance values for the main effects "Genotype" and "Replicate" and the interaction, for the y-variate the logarithm of the RIF. The second part consists of the Dunnetts post-hoc test indicating whether SusPtrit and any other genotype are significant different. The third part contains the same Dunnett post-hoc test, the difference being that as a control group SusQ11 is taken. The genotypes are denoted by codes: 1=homorecombinant 1, 2= homorecombinant 2 etc. α=0.05.

Tests of Between-Subjects Effects

Dependent Variable: logRIF

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	66.042[a]	78	.847	17.211	.000
Intercept	460.994	1	460.994	9370.991	.000
Replicate	7.880	3	2.627	53.397	.000
Genotype	39.099	23	1.700	34.556	.000
Replicate * Genotype	9.513	52	.183	3.719	.000
Error	8.461	172	.049		
Total	577.133	251			
Corrected Total	74.504	250			

a. R Squared = .886 (Adjusted R Squared = .835)

Multiple Comparisons

Dependent Variable: logRIF

Dunnett t (2-sided)

(I) !Genotype	(J) !Genotype	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
SusQ	SusP	-.98677*	.077289	.000	-1.22023	-.75331
1	SusP	-.25774*	.082422	.037	-.50670	-.00877
2	SusP	-.95482*	.098301	.000	-1.25175	-.65789
3	SusP	-.09707	.098301	.998	-.39400	.19986
4	SusP	-.70657*	.081169	.000	-.95175	-.46139
5	SusP	-.17870	.098301	.631	-.47563	.11823
6	SusP	-.24774	.082422	.052	-.49670	.00123
7	SusP	-.16895	.098301	.710	-.46588	.12798
8	SusP	-.15582	.098301	.809	-.45275	.14111
9	SusP	-.17507	.098301	.661	-.47200	.12186
10	SusP	-.14895	.098301	.855	-.44588	.14798
11	SusP	-.02645	.098301	1.000	-.32338	.27048
12	SusP	-.94057*	.098301	.000	-1.23750	-.64364
13	SusP	-.69382*	.098301	.000	-.99075	-.39689
14	SusP	-.72645*	.098301	.000	-1.02338	-.42952
15	SusP	-.96432*	.098301	.000	-1.26125	-.66739
16	SusP	-.75107*	.098301	.000	-1.04800	-.45414
17	SusP	-.68082*	.098301	.000	-.97775	-.38389
18	SusP	-.66195*	.081169	.000	-.90713	-.41676
19	SusP	-.86095*	.098301	.000	-1.15788	-.56402

20	SusP	-.08182	.098301	1.000	-.37875	.21511
Step	SusP	-1.54291*	.078122	.000	-1.77889	-1.30694
13'	SusP	-.86895*	.098301	.000	-1.16588	-.57202

Based on observed means.

The error term is Mean Square(Error) = .049.

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Multiple Comparisons

Dependent Variable: logRIF

Dunnett t (2-sided)

(I) !Genotype	(J) !Genotype	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	SusQ	.72903*	.075758	.000	.49780	.96027
2	SusQ	.03195	.092784	1.000	-.25126	.31516
3	SusQ	.88970*	.092784	.000	.60649	1.17291
4	SusQ	.28020*	.074393	.005	.05313	.50727
5	SusQ	.80807*	.092784	.000	.52487	1.09128
6	SusQ	.73903*	.075758	.000	.50780	.97027
7	SusQ	.81782*	.092784	.000	.53462	1.10103
8	SusQ	.83095*	.092784	.000	.54774	1.11416
9	SusQ	.81170*	.092784	.000	.52849	1.09491
10	SusQ	.83782*	.092784	.000	.55462	1.12103
11	SusQ	.96033*	.092784	.000	.67712	1.24353
12	SusQ	.04620	.092784	1.000	-.23701	.32941
13	SusQ	.29295*	.092784	.037	.00974	.57616
14	SusQ	.26032	.092784	.099	-.02288	.54353
15	SusQ	.02245	.092784	1.000	-.26076	.30566
16	SusQ	.23570	.092784	.190	-.04751	.51891
17	SusQ	.30595*	.092784	.024	.02274	.58916
18	SusQ	.32482*	.074393	.000	.09775	.55190
19	SusQ	.12583	.092784	.957	-.15738	.40903
20	SusQ	.90495*	.092784	.000	.62174	1.18816
Step	SusQ	-.55614*	.071055	.000	-.77303	-.33926
13'	SusQ	.11782	.092784	.977	-.16538	.40103
SusP	SusQ	.98677*	.077289	.000	.75086	1.22268

Based on observed means.

The error term is Mean Square(Error) = .049.

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table 39. SPSS output for the first part of the substitution mapping procedure of *Pt*. This table consists of 3 parts, namely an ANOVA table showing the significance values for the main effects "Genotype" and "Replicate" and the interaction, for the y-variate the logarithm of the RIF. The second part consists of the Dunnetts post-hoc test indicating whether SusPtrit and any other genotype are significant different. The third part contains the same Dunnett post-hoc test, the difference being that as a control group SusQ11 is taken. The genotypes are denoted by codes: 1=homorecombinant 1, 2= homorecombinant 2 etc. α=0.05.

Tests of Between-Subjects Effects

Dependent Variable: logRIF

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	43.212[a]	71	.609	29.644	.000
Intercept	464.039	1	464.039	22601.524	.000
Genotype	28.972	23	1.260	61.353	.000
Replicate	.474	2	.237	11.548	.000
Genotype * Replicate	2.487	46	.054	2.633	.000
Error	2.587	126	.021		
Total	546.299	198			
Corrected Total	45.799	197			

a. R Squared = .944 (Adjusted R Squared = .912)

Multiple Comparisons

Dependent Variable: logRIF

Dunnett t (2-sided)

(I) !Genotype	(J) !Genotype	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
SusQ	SusP	-.493988*	.0654015	.000	-.689305	-.298670
1	SusP	-.117450	.0716438	.699	-.331410	.096510
2	SusP	-.547300*	.0716438	.000	-.761260	-.333340
3	SusP	-.007725	.0716438	1.000	-.221685	.206235
4	SusP	-.527450*	.0716438	.000	-.741410	-.313490
5	SusP	-.074213	.0716438	.990	-.288172	.139747
6	SusP	-.185829	.0773841	.201	-.416932	.045274
7	SusP	-.193688	.0716438	.103	-.407647	.020272
8	SusP	-.032512	.0716438	1.000	-.246472	.181447
9	SusP	-.105563	.0716438	.821	-.319522	.108397
10	SusP	-.079374	.0696252	.973	-.287305	.128558
11	SusP	.051712	.0716438	1.000	-.162247	.265672
12	SusP	-.435900*	.0716438	.000	-.649860	-.221940
13	SusP	-.368775*	.0716438	.000	-.582735	-.154815
14	SusP	-.390637*	.0716438	.000	-.604597	-.176678
15	SusP	-.537163*	.0716438	.000	-.751122	-.323203
16	SusP	-.373175*	.0716438	.000	-.587135	-.159215
17	SusP	-.504388*	.0716438	.000	-.718347	-.290428
18	SusP	-.544413*	.0716438	.000	-.758372	-.330453
19	SusP	-.781662*	.0716438	.000	-.995622	-.567703

20	SusP	-.014134	.0741583	1.000	-.235603	.207336
Step	SusP	-1.936421*	.0654015	.000	-2.131739	-1.741103
13'	SusP	-.428100*	.0716438	.000	-.642060	-.214140

Based on observed means.

The error term is Mean Square(Error) = .021.

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Multiple Comparisons

Dependent Variable: logRIF

Dunnett t (2-sided)

(I) !Genotype	(J) !Genotype	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	SusQ	.376537*	.0654015	.000	.177806	.575269
2	SusQ	-.053313	.0654015	1.000	-.252044	.145419
3	SusQ	.486263*	.0654015	.000	.287531	.684994
4	SusQ	-.033462	.0654015	1.000	-.232194	.165269
5	SusQ	.419775*	.0654015	.000	.221044	.618506
6	SusQ	.308158*	.0716438	.001	.090459	.525857
7	SusQ	.300300*	.0654015	.000	.101569	.499031
8	SusQ	.461475*	.0654015	.000	.262744	.660206
9	SusQ	.388425*	.0654015	.000	.189694	.587156
10	SusQ	.414614*	.0631839	.000	.222621	.606606
11	SusQ	.545700*	.0654015	.000	.346969	.744431
12	SusQ	.058087	.0654015	.999	-.140644	.256819
13	SusQ	.125212	.0654015	.559	-.073519	.323944
14	SusQ	.103350	.0654015	.817	-.095381	.302081
15	SusQ	-.043175	.0654015	1.000	-.241906	.155556
16	SusQ	.120812	.0654015	.613	-.077919	.319544
17	SusQ	-.010400	.0654015	1.000	-.209131	.188331
18	SusQ	-.050425	.0654015	1.000	-.249156	.148306
19	SusQ	-.287675*	.0654015	.000	-.486406	-.088944
20	SusQ	.479854*	.0681468	.000	.272780	.686927
Step	SusQ	-1.442433*	.0584969	.000	-1.620184	-1.264683
13'	SusQ	.065888	.0654015	.997	-.132844	.264619
SusP	SusQ	.493988*	.0654015	.000	.295256	.692719

Based on observed means.

The error term is Mean Square(Error) = .021.

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table 40. SPSS output for the first part of the substitution mapping procedure of *Pgl*. This table consists of 3 parts, namely an ANOVA table showing the significance values for the main effects "Genotype" and "Replicate" and the interaction, for the y-variate the logarithm of the RIF. The second part consists of the Dunnetts post-hoc test indicating whether SusPtrit and any other genotype are significant different. The third part contains the same Dunnett post-hoc test, the difference being that as a control group SusQ11 is taken. The genotypes are denoted by codes: 1=homorecombinant 1, 2= homorecombinant 2 etc. α=0.05.

Tests of Between-Subjects Effects

Dependent Variable: logRIF

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	23.220[a]	78	.298	14.131	.000
Intercept	561.298	1	561.298	26644.179	.000
Genotype	17.363	23	.755	35.835	.000
Replicate	.196	3	.065	3.104	.028
Genotype * Replicate	4.573	52	.088	4.174	.000
Error	3.118	148	.021		
Total	738.629	227			
Corrected Total	26.337	226			

a. R Squared = .882 (Adjusted R Squared = .819)

Multiple Comparisons

Dependent Variable: logRIF

Dunnett t (2-sided)

(I) !Genotype	(J) !Genotype	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
SusQ	SusP	-.4610283*	.05542733	.000	-.6283573	-.2936994
1	SusP	-.0468071	.06624834	1.000	-.2468035	.1531893
2	SusP	-.2306961*	.06058604	.004	-.4135986	-.0477935
3	SusP	.1257179	.06624834	.554	-.0742785	.3257143
4	SusP	-.1011783	.05709888	.655	-.2735535	.0711969
5	SusP	.2585617*	.06624834	.003	.0585653	.4585581
6	SusP	-.0839471	.06624834	.959	-.2839435	.1160493
7	SusP	.0773717	.06624834	.981	-.1226247	.2773681
8	SusP	.1123179	.06624834	.716	-.0876785	.3123143
9	SusP	-.0227962	.06902919	1.000	-.2311877	.1855953
10	SusP	.0172392	.06624834	1.000	-.1827572	.2172356
11	SusP	.0747724	.06902919	.991	-.1336191	.2831638
12	SusP	-.4755048*	.06902919	.000	-.6838962	-.2671133
13	SusP	-.4043808*	.06624834	.000	-.6043772	-.2043844
14	SusP	-.1772608	.06624834	.122	-.3772572	.0227356
15	SusP	-.4019083*	.06624834	.000	-.6019047	-.2019119
16	SusP	-.3420421*	.06624834	.000	-.5420385	-.1420457
17	SusP	-.2667771*	.06624834	.002	-.4667735	-.0667807
18	SusP	-.3534558*	.05542733	.000	-.5207848	-.1861269
19	SusP	-.2545083*	.06624834	.004	-.4545047	-.0545119

20	SusP	.0241233	.05925431	1.000	-.1547589	.2030055
Step	SusP	-.9616527*	.05621358	.000	-1.1313552	-.7919501
13'	SusP	-.2809733*	.07257142	.003	-.5000584	-.0618883

Based on observed means.

The error term is Mean Square(Error) = .021.

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Multiple Comparisons

Dependent Variable: logRIF

Dunnett t (2-sided)

(I) !Genotype	(J) !Genotype	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	SusQ	.4142213*	.06284869	.000	.2226695	.6057730
2	SusQ	.2303323*	.05684878	.002	.0570672	.4035974
3	SusQ	.5867463*	.06284869	.000	.3951945	.7782980
4	SusQ	.3598500*	.05311684	.000	.1979592	.5217408
5	SusQ	.7195900*	.06284869	.000	.5280382	.9111418
6	SusQ	.3770813*	.06284869	.000	.1855295	.5686330
7	SusQ	.5384000*	.06284869	.000	.3468482	.7299518
8	SusQ	.5733463*	.06284869	.000	.3817945	.7648980
9	SusQ	.4382321*	.06577343	.000	.2377662	.6386980
10	SusQ	.4782675*	.06284869	.000	.2867157	.6698193
11	SusQ	.5358007*	.06577343	.000	.3353348	.7362666
12	SusQ	-.0144764	.06577343	1.000	-.2149423	.1859895
13	SusQ	.0566475	.06284869	1.000	-.1349043	.2481993
14	SusQ	.2837675*	.06284869	.000	.0922157	.4753193
15	SusQ	.0591200	.06284869	.999	-.1324318	.2506718
16	SusQ	.1189863	.06284869	.605	-.0725655	.3105380
17	SusQ	.1942513*	.06284869	.044	.0026995	.3858030
18	SusQ	.1075725	.05131574	.445	-.0488289	.2639739
19	SusQ	.2065200*	.06284869	.025	.0149682	.3980718
20	SusQ	.4851517*	.05542733	.000	.3162189	.6540845
Step	SusQ	-.5006243*	.05216399	.000	-.6596110	-.3416376
13'	SusQ	.1800550	.06948184	.164	-.0317135	.3918235
SusP	SusQ	.4610283*	.05542733	.000	.2920955	.6299611

Based on observed means.

The error term is Mean Square(Error) = .021.

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table 41. SPSS output for the first part of the substitution mapping procedure of *Phm*. This table consists of 3 parts, namely an ANOVA table showing the significance values for the main effects "Genotype" and "Replicate" and the interaction, for the y-variate the logarithm of the RIF. The second part consists of the Dunnetts post-hoc test indicating whether SusPtrit and any other genotype are significant different. The third part contains the same Dunnett post-hoc test, the difference being that as a control group SusQ11 is taken. The genotypes are denoted by codes: 1=homorecombinant 1, 2= homorecombinant 2 etc. α=0.05.

Tests of Between-Subjects Effects

Dependent Variable: logRIF

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	42.495[a]	71	.599	30.997	.000
Intercept	410.991	1	410.991	21285.031	.000
Replicate	.735	2	.367	19.027	.000
Genotype	28.635	23	1.245	64.478	.000
Replicate * Genotype	3.493	46	.076	3.933	.000
Error	2.433	126	.019		
Total	511.228	198			
Corrected Total	44.927	197			

a. R Squared = .946 (Adjusted R Squared = .915)

Multiple Comparisons

Dependent Variable: logRIF

Dunnett t (2-sided)

(I) !Genotype	(J) !Genotype	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
SusQ	SusP	-.6403149*	.06245632	.000	-.8278777	-.4527522
1	SusP	-.2945010*	.06752074	.001	-.4972727	-.0917292
2	SusP	-.5250660*	.06752074	.000	-.7278377	-.3222942
3	SusP	-.0673622	.06752074	.995	-.2701340	.1354096
4	SusP	-.7375960*	.06752074	.000	-.9403677	-.5348242
5	SusP	-.1639908	.07002751	.239	-.3742907	.0463091
6	SusP	-.2300672*	.06752074	.015	-.4328390	-.0272954
7	SusP	-.1333935	.06752074	.462	-.3361652	.0693783
8	SusP	-.1308235	.06752074	.490	-.3335952	.0719483
9	SusP	-.1195872	.06752074	.619	-.3223590	.0831846
10	SusP	-.1394372	.06752074	.399	-.3422090	.0633346
11	SusP	-.0749779	.07002751	.989	-.2852778	.1353219
12	SusP	-.4947260*	.06752074	.000	-.6974977	-.2919542
13	SusP	-.6029735*	.06752074	.000	-.8057452	-.4002017
14	SusP	-.4904710*	.06752074	.000	-.6932427	-.2876992
15	SusP	-.5975060*	.06752074	.000	-.8002777	-.3947342
16	SusP	-.4374447*	.06752074	.000	-.6402165	-.2346729
17	SusP	-.4953860*	.06752074	.000	-.6981577	-.2926142
18	SusP	-.4407135*	.06752074	.000	-.6434852	-.2379417
19	SusP	-.5530447*	.06752074	.000	-.7558165	-.3502729

20	SusP	-.1263097	.06752074	.541	-.3290815	.0764621
Step	SusP	-1.9482622*	.06127407	.000	-2.1322746	-1.7642498
13'	SusP	-.6822735*	.06752074	.000	-.8850452	-.4795017

Based on observed means.

The error term is Mean Square(Error) = .019.

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Multiple Comparisons

Dependent Variable: logRIF

Dunnett t (2-sided)

(I) !Genotype	(J) !Genotype	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	SusQ	.3458140*	.06456757	.000	.1502609	.5413671
2	SusQ	.1152490	.06456757	.647	-.0803041	.3108021
3	SusQ	.5729527*	.06456757	.000	.3773997	.7685058
4	SusQ	-.0972810	.06456757	.851	-.2928341	.0982721
5	SusQ	.4763242*	.06718462	.000	.2728449	.6798034
6	SusQ	.4102477*	.06456757	.000	.2146947	.6058008
7	SusQ	.5069215*	.06456757	.000	.3113684	.7024746
8	SusQ	.5094915*	.06456757	.000	.3139384	.7050446
9	SusQ	.5207277*	.06456757	.000	.3251747	.7162808
10	SusQ	.5008777*	.06456757	.000	.3053247	.6964308
11	SusQ	.5653370*	.06718462	.000	.3618578	.7688162
12	SusQ	.1455890	.06456757	.307	-.0499641	.3411421
13	SusQ	.0373415	.06456757	1.000	-.1582116	.2328946
14	SusQ	.1498440	.06456757	.270	-.0457091	.3453971
15	SusQ	.0428090	.06456757	1.000	-.1527441	.2383621
16	SusQ	.2028702*	.06456757	.036	.0073172	.3984233
17	SusQ	.1449290	.06456757	.313	-.0506241	.3404821
18	SusQ	.1996015*	.06456757	.042	.0040484	.3951546
19	SusQ	.0872702	.06456757	.930	-.1082828	.2828233
20	SusQ	.5140052*	.06456757	.000	.3184522	.7095583
Step	SusQ	-1.3079473*	.05800373	.000	-1.4836207	-1.1322738
13'	SusQ	-.0419585	.06456757	1.000	-.2375116	.1535946
SusP	SusQ	.6403149*	.06245632	.000	.4511561	.8294738

Based on observed means.

The error term is Mean Square(Error) = .019.

*. The mean difference is significant at the .05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

7.1.5 Histological assessment

Table 42. Phenotypic results of the pathogen *Phb_Iran* of the IF evaluation for the plants used in the histological assessment. Length and width of each measured leaf is given in cm. In addition, for each leaf the counted number of pustules is given. Colors indicate highest and lowest RIF values, from dark green (lowest) to dark red (highest).

Pathogen	Genotype	Plant number	Pustules	Length	Width	IF	Average	RIF
Phb_Iran	SusPtrit	1	460	17.1	0.8	33.6	26.6	100.0
Phb_Iran	SusPtrit	2	260	15.1	0.7	24.6		
Phb_Iran	SusPtrit	3	290	15.3	0.8	23.7		
Phb_Iran	SusPtrit	4	190	9.7	0.75	26.1		
Phb_Iran	SusPtrit	5	310	17.1	0.6	30.2		
Phb_Iran	SusPtrit	6	250	13.1	0.9	21.2		
Phb_Iran	Steptoe	1	14	16.1	0.9	1.0	1.3	5.0
Phb_Iran	Steptoe	2	14	17.1	0.9	0.9		
Phb_Iran	Steptoe	3	21	15.7	0.9	1.5		
Phb_Iran	Steptoe	4	21	15.9	0.8	1.7		
Phb_Iran	Steptoe	5	18	16.6	0.8	1.4		
Phb_Iran	Steptoe	6	22	16.5	0.85	1.6		
Phb_Iran	SusQ11	1	31	12	0.65	4.0	2.8	10.6
Phb_Iran	SusQ11	2	19	13	0.6	2.4		
Phb_Iran	SusQ11	3	16	12	0.45	3.0		
Phb_Iran	SusQ11	4	13	12.6	0.5	2.1		
Phb_Iran	SusQ11	5	19	12.6	0.5	3.0		
Phb_Iran	SusQ11	6	14	11.3	0.5	2.5		
Phb_Iran	Rec7.	1	127	14.8	0.7	12.3	16.6	62.5
Phb_Iran	Rec7.	2	159	12.8	0.6	20.7		
Phb_Iran	Rec7.	3	181	14	0.6	21.5		
Phb_Iran	Rec7.	4	151	14.3	0.75	14.1		
Phb_Iran	Rec7.	5	143	13.5	0.6	17.7		
Phb_Iran	Rec7.	6	132	14	0.7	13.5		
Phb_Iran	Rec19.	1	31	16.7	0.65	2.9	3.2	12.2
Phb_Iran	Rec19.	2	47	16.5	0.65	4.4		
Phb_Iran	Rec19.	3	34	14.2	0.6	4.0		
Phb_Iran	Rec19.	4	23	16.2	0.7	2.0		
Phb_Iran	Rec19.	5	28	15.1	0.65	2.9		
Phb_Iran	Rec19.	6	24	14.7	0.5	3.3		

Table 43. Overview of the number of early abortions (EA) with (+N) and without (-N) necrosis and the number of established (EST) infections with (+N) and without (-N) necrosis for each of the assessed leaves. Counting's add up to a total of 50 units. The number of established infections with sporogenic tissue is given in the last column. Sample numbers are constructed by "genotype" – "slide number" – "leaf number". On each slide, two leaves were present.

Sample	EA-N	EA+N	EST-N	EST+N	EST sporogeen
St-1-1	35	2	13	0	0
St-1-2	38	2	10	0	0
St-2-1	32	0	18	0	0
St-2-2	35	0	14	1	0
SusP-1-1	6	0	44	0	28
SusP-1-2	14	1	35	0	16
SusP-2-1	11	0	38	1	17
SusP-2-2	11	1	38	0	15
SusQ-1-1	15	1	35	0	0
SusQ-1-2	27	0	22	1	0
SusQ-2-1	12	1	37	0	0
SusQ-2-2	24	0	26	0	0
Rec7-1-1	13	2	35	0	9
Rec7-1-2	10	2	37	1	13
Rec7-2-1	10	0	40	0	7
Rec7-2-2	10	0	40	0	9
Rec19-1-1	25	4	20	1	0
Rec19-1-2	14	3	32	1	2
Rec19-2-1	20	1	29	0	0
Rec19-2-2	18	1	31	1	0

Table 44. Width of each individual established infection (EST) with (+N) and without (-N) necrosis on the assessed leaves.

Sample	EST-N	EST+N	Sample	EST-N	EST+N	Sample	EST-N	EST+N	Sample	EST-N	EST+N
St-1-1	1.4		St-1-2	2		St-2-1	5.9		St-2-2	2.7	2
St-1-1	3.2		St-1-2	3.3		St-2-1	3.2		St-2-2	2.1	
St-1-1	2.9		St-1-2	1.8		St-2-1	4.1		St-2-2	5.4	
St-1-1	2.4		St-1-2	2.7		St-2-1	2.9		St-2-2	5.2	
St-1-1	2.8		St-1-2	3		St-2-1	1.9		St-2-2	2.8	
St-1-1	2.2		St-1-2	3.3		St-2-1	3.5		St-2-2	2	
St-1-1	1.7		St-1-2	1.8		St-2-1	3.2		St-2-2	1.5	
St-1-1	2.1		St-1-2	4.6		St-2-1	3		St-2-2	2.5	
St-1-1	1.6		St-1-2	2.5		St-2-1	2.5		St-2-2	3.9	
St-1-1	2.5		St-1-2	3.2		St-2-1	1.1		St-2-2	2.7	
St-1-1	2.9		St-1-2	9.5		St-2-1	3		St-2-2	2.7	
St-1-1	2.7		St-1-2	1.2		St-2-1	2		St-2-2	2.2	
St-1-1	3.2		SusP-1-2	8.8		St-2-1	4.2		St-2-2	1.5	
St-1-1	1.6		SusP-1-2	12.2		St-2-1	2.5		St-2-2	4.3	
St-1-1	1.8		SusP-1-2	7.5		St-2-1	1.4		SusP-2-2	6.5	
St-1-1	1.6		SusP-1-2	2.9		St-2-1	5.5		SusP-2-2	6	
SusP-1-1	7.5		SusP-1-2	4.8		St-2-1	4		SusP-2-2	5.1	
SusP-1-1	7		SusP-1-2	9		SusP-2-1	8.5		SusP-2-2	9.1	
SusP-1-1	6.3		SusP-1-2	5		SusP-2-1	10.2		SusP-2-2	4.3	
SusP-1-1	3.5		SusP-1-2	3.6		SusP-2-1	3.8		SusP-2-2	11	
SusP-1-1	6.7		SusP-1-2	9.5		SusP-2-1	2.3		SusP-2-2	5.8	
SusP-1-1	2.3		SusP-1-2	9.8		SusP-2-1	6.7		SusP-2-2	9	
SusP-1-1	10		SusP-1-2	9.3		SusP-2-1	6.2		SusP-2-2	7.7	
SusP-1-1	8.2		SusP-1-2	5.2		SusP-2-1	9.2		SusP-2-2	8.7	
SusP-1-1	3.6		SusP-1-2	2.5		SusP-2-1	2		SusP-2-2	4.8	
SusP-1-1	9.2		SusP-1-2	5.5		SusP-2-1	10.3		SusP-2-2	4.1	
SusP-1-1	13.7		SusP-1-2	7		SusP-2-1	6.1		SusP-2-2	8.5	
SusP-1-1	12.8		SusP-1-2	12		SusP-2-1	6		SusP-2-2	5	
SusP-1-1	6.5		SusP-1-2	7.9		SusP-2-1	1.8		SusP-2-2	11.5	
SusP-1-1	8.2		SusP-1-2	3.9		SusP-2-1	4.5		SusP-2-2	5.8	
SusP-1-1	4.6		SusP-1-2	4.7		SusP-2-1	6.5		SusP-2-2	6.5	
SusP-1-1	7.2		SusP-1-2	2.3		SusP-2-1	8.5		SusP-2-2	6	
SusP-1-1	11.3		SusP-1-2	1.9		SusP-2-1	11.4		SusP-2-2	5	
SusP-1-1	12.1		SusP-1-2	5.8		SusP-2-1	6.8		SusP-2-2	8.3	
SusP-1-1	10.3		SusP-1-2	2.5		SusP-2-1	6		SusP-2-2	3.3	
SusP-1-1	13		SusP-1-2	8.2		SusP-2-1	6.7		SusP-2-2	10.3	
SusP-1-1	11.2		SusP-1-2	10.6		SusP-2-1	11.1		SusP-2-2	7.9	
SusP-1-1	8.2		SusP-1-2	4.2		SusP-2-1	11.6		SusP-2-2	6	
SusP-1-1	9.6		SusP-1-2	5		SusP-2-1	9.8		SusP-2-2	4.5	
SusP-1-1	7.4		SusP-1-2	4		SusP-2-1	8.9		SusP-2-2	4.5	

SusP-1-1	6.9		SusP-1-2	12.8		SusP-2-1	13.5		SusP-2-2	4.7
SusP-1-1	10.7		SusP-1-2	10.2		SusQ-2-1	3		SusP-2-2	9
SusP-1-1	8.1		SusP-1-2	10.3		SusQ-2-1	5.2		SusQ-2-2	3.2
SusP-1-1	6		SusP-1-2	4		SusQ-2-1	5.3		SusQ-2-2	4.2
SusP-1-1	1.5		SusQ-1-2	3.2		SusQ-2-1	4.9		SusQ-2-2	2.2
SusP-1-1	6		SusQ-1-2	2		SusQ-2-1	5.1		SusQ-2-2	6.3
SusP-1-1	3.3		SusQ-1-2	3.6		SusQ-2-1	6		SusQ-2-2	3.5
SusQ-1-1	2.7		SusQ-1-2	4.2		SusQ-2-1	7.8		SusQ-2-2	3.7
SusQ-1-1	2.5		SusQ-1-2	4.3		SusQ-2-1	2.8		SusQ-2-2	5.4
SusQ-1-1	4.4		SusQ-1-2	4.2		SusQ-2-1	4		SusQ-2-2	4.1
SusQ-1-1	5.2		SusQ-1-2	4.4		SusQ-2-1	1.8		SusQ-2-2	2
SusQ-1-1	3.4		SusQ-1-2	4.7		SusQ-2-1	2.7		SusQ-2-2	2.6
SusQ-1-1	4.2		SusQ-1-2	3.8		SusQ-2-1	3.7		SusQ-2-2	2.3
SusQ-1-1	3.5		SusQ-1-2	3.4		SusQ-2-1	3.7		SusQ-2-2	4.5
SusQ-1-1	3.8		SusQ-1-2	5.1		SusQ-2-1	2.7		SusQ-2-2	7.1
SusQ-1-1	5.5		SusQ-1-2	5.1		SusQ-2-1	2.1		SusQ-2-2	4.3
SusQ-1-1	6.7		SusQ-1-2	6.2		SusQ-2-1	4.5		SusQ-2-2	2.5
SusQ-1-1	3.9		SusQ-1-2	3.7		SusQ-2-1	3.7		SusQ-2-2	3
SusQ-1-1	5.2		SusQ-1-2	3.4		SusQ-2-1	5.4		SusQ-2-2	4.5
SusQ-1-1	2		SusQ-1-2	7		SusQ-2-1	4.1		SusQ-2-2	3.6
SusQ-1-1	4.3		SusQ-1-2	4.1		SusQ-2-1	4.8		SusQ-2-2	1.7
SusQ-1-1	1.7		SusQ-1-2	1.9		SusQ-2-1	4.3		SusQ-2-2	6.2
SusQ-1-1	6.7		SusQ-1-2	4.1		SusQ-2-1	2.7		SusQ-2-2	2.8
SusQ-1-1	5.9		SusQ-1-2	2.7		SusQ-2-1	3.5		SusQ-2-2	4.1
SusQ-1-1	4		SusQ-1-2	1.7		SusQ-2-1	2.6		SusQ-2-2	4.6
SusQ-1-1	4.1		SusQ-1-2	4.1		SusQ-2-1	5.2		SusQ-2-2	2
SusQ-1-1	5.5		Rec7-1-2	8.5		SusQ-2-1	3		SusQ-2-2	3
SusQ-1-1	2		Rec7-1-2	4.2		SusQ-2-1	2.5		SusQ-2-2	3.6
SusQ-1-1	4.6		Rec7-1-2	5.5		SusQ-2-1	2.5		SusQ-2-2	1.3
SusQ-1-1	5		Rec7-1-2	3.5		SusQ-2-1	3.6		SusQ-2-2	3.7
SusQ-1-1	2.3		Rec7-1-2	8		Rec7-2-1	6.1		SusQ-2-2	4
SusQ-1-1	2.5		Rec7-1-2	5.5		Rec7-2-1	9.1		SusQ-2-2	3.8
SusQ-1-1	1.8		Rec7-1-2	5.7		Rec7-2-1	1.8		Rec7-2-2	7
SusQ-1-1	4.7		Rec7-1-2	5.7		Rec7-2-1	7.6		Rec7-2-2	4.6
SusQ-1-1	5.5		Rec7-1-2	7.6		Rec7-2-1	9.6		Rec7-2-2	8.1

SusQ-1-1	1.4		Rec7-1-2	6.5		Rec7-2-1	7.7	Rec7-2-2	6.7
Rec7-1-1	7.8		Rec7-1-2	5		Rec7-2-1	9.5	Rec7-2-2	11
Rec7-1-1	4.2		Rec7-1-2	6.2		Rec7-2-1	3.7	Rec7-2-2	7.7
Rec7-1-1	5.3		Rec7-1-2	7.7		Rec7-2-1	5.2	Rec7-2-2	3.6
Rec7-1-1	3.8		Rec7-1-2	7.1		Rec7-2-1	6.7	Rec7-2-2	9.6
Rec7-1-1	7.4		Rec7-1-2	5.3		Rec7-2-1	5.5	Rec7-2-2	5.6
Rec7-1-1	5.6		Rec7-1-2	6		Rec7-2-1	7	Rec7-2-2	6.5
Rec7-1-1	5.2		Rec7-1-2	3.7		Rec7-2-1	8	Rec7-2-2	9.2
Rec7-1-1	4.1		Rec7-1-2	6.5		Rec7-2-1	5	Rec7-2-2	4.6
Rec7-1-1	5.4		Rec7-1-2	8		Rec7-2-1	6.1	Rec7-2-2	4.2
Rec7-1-1	7.5		Rec7-1-2	8.2		Rec7-2-1	7.9	Rec7-2-2	12.6
Rec7-1-1	6.5		Rec7-1-2	3.7		Rec7-2-1	1.2	Rec7-2-2	5.2
Rec7-1-1	4.4		Rec7-1-2	9.5		Rec7-2-1	7.3	Rec7-2-2	2.3
Rec7-1-1	5.2		Rec7-1-2	7.5		Rec7-2-1	6	Rec7-2-2	8.4
Rec7-1-1	5.7		Rec7-1-2	7.7		Rec7-2-1	9.3	Rec7-2-2	3.6
Rec7-1-1	7.5		Rec19-1-2	2.2	3.6	Rec7-2-1	7.1	Rec7-2-2	9.6
Rec7-1-1	7.1		Rec19-1-2	5.1		Rec7-2-1	9.2	Rec7-2-2	2.9
Rec7-1-1	4.5		Rec19-1-2	3.2		Rec7-2-1	10	Rec7-2-2	7.2
Rec7-1-1	5.8		Rec19-1-2	9.2		Rec7-2-1	8.5	Rec7-2-2	7
Rec7-1-1	4		Rec19-1-2	4.2		Rec7-2-1	6.1	Rec7-2-2	8.3
Rec7-1-1	6.5		Rec19-1-2	5.4		Rec7-2-1	3.1	Rec7-2-2	5.5
Rec7-1-1	9		Rec19-1-2	3.5		Rec7-2-1	7.8	Rec7-2-2	13.2
Rec7-1-1	8.5		Rec19-1-2	3.6		Rec7-2-1	2.5	Rec19-2-2	3.3
Rec7-1-1	3.7		Rec19-1-2	3.5		Rec7-2-1	6	Rec19-2-2	4.5
Rec7-1-1	9.5		Rec19-1-2	6.7		Rec19-2-1	5.1	Rec19-2-2	4.4
Rec7-1-1	7.9		Rec19-1-2	3.6		Rec19-2-1	1.2	Rec19-2-2	6.4
Rec7-1-1	8.2		Rec19-1-2	6.8		Rec19-2-1	2.6	Rec19-2-2	3
Rec19-1-1	5.3	2.2	Rec19-1-2	5.3		Rec19-2-1	6.2	Rec19-2-2	4
Rec19-1-1	3.2		Rec19-1-2	3.7		Rec19-2-1	3.3	Rec19-2-2	3.5
Rec19-1-1	2.6		Rec19-1-2	2.4		Rec19-2-1	4.6	Rec19-2-2	1.4
Rec19-1-1	2.8		Rec19-1-2	4.6		Rec19-2-1	3.7	Rec19-2-2	4
Rec19-1-1	3.5		Rec19-1-2	5.5		Rec19-2-1	5.1	Rec19-2-2	6.4
Rec19-1-1	3		Rec19-1-2	2.1		Rec19-2-1	4.5	Rec19-2-2	7

Rec19-1-1	3.9		Rec19-1-2	4.1		Rec19-2-1	3.8	Rec19-2-2	1.4
Rec19-1-1	3.4		Rec19-1-2	4.1		Rec19-2-1	2.4	Rec19-2-2	4.3
Rec19-1-1	4.2		Rec19-1-2	5.4		Rec19-2-1	7.3	Rec19-2-2	5.8
Rec19-1-1	6.8		Rec19-1-2	3.7		Rec19-2-1	3.2	Rec19-2-2	3
Rec19-1-1	2.6		Rec19-1-2	2.8		Rec19-2-1	5.1	Rec19-2-2	4.3
Rec19-1-1	3.1		Rec19-1-2	13.6		Rec19-2-1	3.7	Rec19-2-2	5.5
Rec19-1-1	2		Rec19-1-2	5.2		Rec19-2-1	2.9	Rec19-2-2	3.7
Rec19-1-1	6.1		Rec19-1-2	2.6		Rec19-2-1	3.9	Rec19-2-2	7.2
Rec19-1-1	5.5		Rec19-1-2	4.5		Rec19-2-1	3.1	Rec19-2-2	3.2
Rec19-1-1	3.5		Rec19-1-2	1.8		Rec19-2-1	4.3	Rec19-2-2	6.2
Rec19-1-1	4.6		Rec19-1-2	4		Rec19-2-1	3.6	Rec19-2-2	6.4
Rec19-1-1	3.5		Rec19-1-2	5.6		Rec19-2-1	2.4	Rec19-2-2	4.2
						Rec19-2-1	6.6	Rec19-2-2	3.6
						Rec19-2-1	4	Rec19-2-2	4
						Rec19-2-1	5.8	Rec19-2-2	3
						Rec19-2-1	3	Rec19-2-2	2.6
						Rec19-2-1	4.5		
						Rec19-2-1	2.5		

7.2 Resistance to *Pp* and *Phb* in L94 background

7.2.1 Genotyping

Table 45. Molecular markers and their respective forward and reverse primers. These markers were used for the identification of heterozygous plant in the progeny of the 1501-2 to 1501-5 parents.

Marker	Locus name	Position on CM (cM)	Forward	Reverse	Source
3_P	**BOPA1_868-675**	123.338	CCTAGATCATAGCACCGTTCG	TTCACGCTGACAAAGTACCG	Chisenga (2013)
7_P	**BOPA1_1381-547**	132.302	AGGGCATCAGCATGGGTA	CAAGATGGAGGTCGAACACA	Chisenga (2013)
8_P	**BOPA1_1381-547**	132.302	AGGGCATCAGCATGGGTAAC	CAAGATGGAGGTCGAACACA	Chisenga (2013)
13_P	**SCRI_RS_225187**	10.620	GATAGGCAGGAAGCCATGAA	GACGGAACAAACCTCTCCAA	Chisenga (2013)
26_P	**SCRI_RS_169728**	60.634	CTCCCCACTTTTCATGGTTG	AGATGACACCCACATCATGC	Chisenga (2013)
36_P	**SCRI_RS_130605**	60.634	CAAAATGTTGGGGGATAGGA	GGCAAGCCTTCTTTAGGAGA	Chisenga (2013)

Table 46. Genotyping results of the progeny of the 1502 to 1505 plants (n=100). Genotyping was performed with LightScanner for marker 3 and 26. Each of these plants was phenotyped with *Pp* (RIF). The plant name ("Plant") is constructed by using the parent name and adding an individual number. L = L94, H = heterozygous, S = SusPtrit, U = unassignable.

Plant	RIF	Marker 3 2H; 123.34	Marker 26 6H; 60.63	Plant	RIF	Marker 3 2H; 123.34	Marker 26 6H; 60.63
L94	100.0	S	S	1501-4_9	2.2	L	H
SusP	2.1	L	L	1501-4_10	6.1	H	H
1501-2_1	13.6	H	S	1501-4_11	12.9	H	H
1501-2_2	30.7	H	S	1501-4_12	18.6	S	L
1501-2_3	11.1	L	H	1501-4_13	13.6	U	H
1501-2_4	32.0	H	L	1501-4_14	52.0	S	H
1501-2_5	0.4	L	L	1501-4_15	2.1	L	H
1501-2_6	6.5	S	L	1501-4_16	22.3	S	H
1501-2_7	18.2	H	H	1501-4_18	18.9	S	L
1501-3_1	15.7	H	H	1501-4_19	5.6	S	H
1501-3_2	26.8	H	H	1501-4_20	20.1	H	H
1501-3_3	9.3	H	L	1501-4_21	9.8	L	U
1501-3_4	13.6	L	L	1501-4_22	23.5	H	H
1501-3_5	8.9	L	U	1501-4_23	9.1	H	H
1501-3_6	23.8	H	H	1501-4_24	2.3	H	H
1501-3_7	53.0	S	H	1501-4_25	5.9	U	U
1501-3_8	8.5	L	L	1501-4_26	0.7	L	L
1501-3_9	12.2	H	U	1501-4_27	3.3	L	L
1501-3_10	56.2	S	H	1501-4_28	4.3	L	L
1501-3_11	15.9	H	H	1501-4_29	25.2	U	L
1501-3_12	24.2	L	H	1501-5_1	4.3	U	H
1501-3_13	22.0	H	H	1501-5_2	47.2	H	H
1501-3_14	4.7	H	H	1501-5_3	35.7	H	H
1501-3_15	34.1	H	H	1501-5_4	15.0	S	H
1501-3_16	28.3	H	H	1501-5_5	1.8	L	L
1501-3_17	9.0	L	H	1501-5_6	14.9	S	L
1501-3_18	13.1	L	U	1501-5_7	0.0	S	L

1501-3_19	6.5	H	L
1501-3_20	0.6	L	H
1501-3_21	97.1	S	H
1501-3_22	4.3	S	L
1501-3_23	18.8	L	H
1501-3_24	8.4	H	L
1501-3_25	55.1	L	H
1501-3_26	6.5	H	H
1501-3_27	10.2	U	L
1501-3_28	32.5	H	H
1501-3_29	4.4	H	L
1501-3_30	45.8	S	H
1501-3_31	6.8	L	H
1501-3_32	23.5	H	H
1501-3_33	6.5	L	L
1501-4_1	4.9	L	U
1501-4_2	19.6	U	L
1501-4_3	40.5	U	H
1501-4_4	3.4	L	H
1501-4_5	1.2	L	L
1501-4_6	8.1	U	H
1501-4_7	99.7	U	U
1501-4_8	48.2	H	H

1501-5_8	29.2	H	L
1501-5_9	85.4	U	U
1501-5_10	70.5	H	H
1501-5_11	13.8	L	H
1501-5_12	1.8	L	L
1501-5_13	3.3	L	L
1501-5_14	51.9	S	S
1501-5_15	6.5	S	S
1501-5_16	15.5	H	H
1501-5_17	9.1	H	L
1501-5_18	28.5	H	L
1501-5_19	34.2	H	H
1501-5_20	18.0	U	U
1501-5_21	5.3	U	H
1501-5_22	43.6	U	H
1501-5_23	61.8	S	L
1501-5_24	24.0	H	H
1501-5_25	33.6	S	H
1501-5_26		H	S
1501-5_27	9.0	S	S
1501-5_28	19.2	H	H
1501-5_29	15.6	L	H
1501-5_30	52.3	H	L

Table 47. Genotyping results of the progeny of the 1502 to 1505 plants (n=70). Genotyping was performed with LightScanner for marker 3 and 26. Each of these plants was phenotyped with *Phb_Isr* (RIF). The plant name ("Plant") is constructed by using the parent name and adding an individual number. L = L94, H = heterozygous, S = SusPtrit, U = unassignable.

Plant	RIF	Marker 3 2H; 123.34	Marker 26 6H; 60.63
1501-2_1	13.3	L	S
1501-2_2	8.3	L	L
1501-2_3	6.9	L	H
1501-2_4	13.1	H	H
1501-2_5	53.7	S	H
1501-2_6	12.2	S	S
1501-2_7	18.2	U	H
1501-2_8	6.7	H	H
1501-2_9	35.7	L	H
1501-2_10	23.8	S	L
1501-2_11	1.3	S	H
1501-2_12	50.8	L	H
1501-2_13	0.0	S	L
1501-2_14	0.0	L	U
1501-2_15	11.7	L	H
1501-2_16	15.7	L	L
1501-2_17	38.2	H	H
1501-2_18	2.5	H	H
1501-2_19	2.7	S	H
1501-2_20	8.3	L	S
1501-2_21	4.0	U	H
1501-2_22	46.5	H	H
1501-2_23	6.2	H	H
1501-2_24	11.4	H	H
1501-2_25	14.5	S	H
1501-2_26	1.8	L	U
1501-2_27	18.1	S	U
1501-2_28	17.6	U	H
1501-2_29	4.3	L	H
1501-2_30	9.4	H	L
1501-2_31	11.4	H	L
1501-2_32	2.6	H	H
1501-2_33	13.6	H	H
1501-2_34	6.0	S	L
1501-5_1	8.1	L	H
1501-5_2	0.0	L	H
1501-5_3	1.5	L	H
1501-5_4	23.8	H	H
1501-5_5	19.9	H	S
1501-5_6	50.7	H	H
1501-5_7	4.5	U	H
1501-5_8	29.7	H	U
1501-5_9	45.0	H	H
1501-5_10	7.9	U	H
1501-5_11	2.0	H	U
1501-5_12	64.9	U	L
1501-5_13	34.4	H	S
1501-5_14	63.6	S	L
1501-5_15	115.0	S	S
1501-5_16	8.8	H	H
1501-5_17	8.0	S	H
1501-5_18	11.1	H	L
1501-5_19	0.7	U	H
1501-5_20	1.3	U	L
1501-5_21	62.9	H	L
1501-5_22	17.2	S	H
1501-5_23	72.5	H	H
1501-5_24	22.8	H	S
1501-5_25	23.4	S	L
1501-5_26	21.2	U	L
1501-5_27	49.9	S	L
1501-5_28	71.4	U	L
1501-5_29	11.3	H	S
1501-5_30	8.5	S	L
1501-5_31	19.1	S	L
1501-5_32	86.0	S	H
1501-5_33	8.1	U	H
1501-5_34	1.1	H	L
1501-5_35	62.4	H	H

7.2.2 Phenotyping

Table 48. Phenotypic results of inoculation with the pathogen *Pp* for the controls SusPtrit (SusP) and L94, for the three boxes inoculated with *Pp*. Width, length and the amount of pustules is given for each individual. Colors indicate highest and lowest value, from dark green (lowest) to dark red (highest).

Pathogen	Genotype	Plant number	Box	Pustules	Length	Width	IF
Pp	SusP	1	1	270	16,8	0,75	21,4
Pp	SusP	2	1	340	14,7	0,8	28,9
Pp	SusP	3	1	320	14,2	0,9	25,0
Pp	SusP	4	1	360	16,6	0,85	25,5
Pp	SusP	5	1	280	12,2	0,7	32,8
Pp	SusP	6	2	215	14,6	0,8	18,4
Pp	SusP	7	2	350	13,8	0,85	29,8
Pp	SusP	8	2	370	15,4	0,8	30,0
Pp	SusP	9	2	250	10,5	0,9	26,5
Pp	SusP	10	3	160	14	0,7	16,3
Pp	SusP	11	3	225	13	0,6	28,8
Pp	SusP	12	3	265	13	0,8	25,5
Pp	SusP	13	3	270	12	0,8	28,1
Pp	L94	1	1	5			0,0
Pp	L94	2	1	3	14,7	0,8	0,3
Pp	L94	3	1	1			0,0
Pp	L94	4	1	17	16	0,9	1,2
Pp	L94	5	1	14	11,7	0,8	1,5
Pp	L94	6	1	8	16	0,9	0,6
Pp	L94	7	2	0			0,0
Pp	L94	8	2	6	17,5	0,9	0,4
Pp	L94	9	2	18	17	0,9	1,2
Pp	L94	10	2	2	15	0,8	0,2
Pp	L94	11	3	5	15	0,9	0,4
Pp	L94	12	3	9	11,4	0,7	1,1
Pp	L94	13	3	12	16	0,95	0,8
Pp	L94	14	3	5	15	0,8	0,4
Pp	L94	15	3	3	16,2	0,9	0,2

Table 49. Phenotypic results of inoculation with the pathogen *Pp* for the progeny of 1501-2 to 1501-5 and the controls SusPtrit (SusP) and L94. Width, length, amount of pustules and the box number is given for each individual. Colors indicate highest and lowest value, from dark green (lowest) to dark red (highest

Pathogen	Genotype	Plant number	Box	Pustules	Length	Width	IF
Pp	1501-3	1	1	35	13,2	0,65	4,1
Pp	1501-3	2	1	33	9,5	0,5	6,9
Pp	1501-3	3	1	18	11,5	0,65	2,4
Pp	1501-3	4	1	28	12,2	0,65	3,5
Pp	1501-3	5	1	28	15,2	0,8	2,3
Pp	1501-3	6	1	69	14	0,8	6,2

Pp	1501-3	7	1	130	12,6	0,75	13,8
Pp	1501-3	8	1	20	12,9	0,7	2,2
Pp	1501-3	9	1	30	12,6	0,75	3,2
Pp	1501-3	10	1	130	11,9	0,75	14,6
Pp	1501-3	11	1	45	13,6	0,8	4,1
Pp	1501-3	12	1	50	11,4	0,7	6,3
Pp	1501-3	13	1	44	11	0,7	5,7
Pp	1501-3	14	1	14	14,4	0,8	1,2
Pp	1501-3	15	1	88	14,2	0,7	8,9
Pp	1501-3	16	1	85	14,5	0,8	7,3
Pp	1501-3	17	1	28	15	0,8	2,3
Pp	1501-3	18	1	27	12,2	0,65	3,4
Pp	1501-3	19	1	16	13,5	0,7	1,7
Pp	1501-3	20	1	2	15,3	0,8	0,2
Pp	1501-3	21	1	240	12,7	0,75	25,2
Pp	1501-3	22	1	9	13,6	0,6	1,1
Pp	1501-3	23	1	60	15,4	0,8	4,9
Pp	1501-3	24	1	26	15	0,8	2,2
Pp	1501-3	25	1	130	13	0,7	14,3
Pp	1501-3	26	1	21	14,6	0,85	1,7
Pp	1501-3	27	1	24	13	0,7	2,6
Pp	1501-3	28	3	40	9,5	0,5	8,4
Pp	1501-3	29	3	5	8,7	0,5	1,1
Pp	1501-3	30	3	68	10,4	0,55	11,9
Pp	1501-3	31	3	8	8,3	0,55	1,8
Pp	1501-3	32	3	25	8,2	0,5	6,1
Pp	1501-3	33	3	15	12,8	0,7	1,7
Pp	1501-4	1	2	18	16,8	0,85	1,3
Pp	1501-4	2	2	39	11,8	0,65	5,1
Pp	1501-4	3	2	93	11,8	0,75	10,5
Pp	1501-4	4	2	10	15	0,75	0,9
Pp	1501-4	5	2	2	10,5	0,6	0,3
Pp	1501-4	6	2	24	14,3	0,8	2,1
Pp	1501-4	7	2	190	10,5	0,7	25,9
Pp	1501-4	8	2	160	16	0,8	12,5
Pp	1501-4	9	2	5	13,2	0,65	0,6
Pp	1501-4	10	2	16	14,5	0,7	1,6
Pp	1501-4	11	2	36	13,5	0,8	3,3
Pp	1501-4	12	2	29	10	0,6	4,8
Pp	1501-4	13	2	35	13,2	0,75	3,5
Pp	1501-4	14	2	170	14	0,9	13,5
Pp	1501-4	15	2	6	14,5	0,75	0,6
Pp	1501-4	16	2	77	14,8	0,9	5,8
Pp	1501-4	17	2	190	14,5	0,85	15,4
Pp	1501-4	18	2	55	14	0,8	4,9
Pp	1501-4	19	2	14	12,8	0,75	1,5

Pp	1501-4	20	2	50	16	0,6	5,2
Pp	1501-4	21	2	31	15,2	0,8	2,5
Pp	1501-4	22	2	88	17	0,85	6,1
Pp	1501-4	23	2	25	14,1	0,75	2,4
Pp	1501-4	24	2	8	15,7	0,85	0,6
Pp	1501-4	25	2	13	12,2	0,7	1,5
Pp	1501-4	26	2	3	17,5	0,9	0,2
Pp	1501-4	27	2	7	13,8	0,6	0,8
Pp	1501-4	28	2	16	15,8	0,9	1,1
Pp	1501-4	29	3	36	11	0,5	6,5
Pp	1501-2	1	3	22	12,5	0,5	3,5
Pp	1501-2	2	3	57	13	0,55	8,0
Pp	1501-2	3	3	34	15,8	0,75	2,9
Pp	1501-2	4	3	54	10	0,65	8,3
Pp	1501-2	5	3	1	13,5	0,7	0,1
Pp	1501-2	6	3	20	15,7	0,75	1,7
Pp	1501-2	7	3	26	10	0,55	4,7
Pp	1501-5	1	3	13	14,7	0,8	1,1
Pp	1501-5	2	3	137	14	0,8	12,2
Pp	1501-5	3	3	79	12,2	0,7	9,3
Pp	1501-5	4	3	59	16,8	0,9	3,9
Pp	1501-5	5	3	6	16,5	0,8	0,5
Pp	1501-5	6	3	38	14	0,7	3,9
Pp	1501-5	7	3	0			0,0
Pp	1501-5	8	3	61	11,5	0,7	7,6
Pp	1501-5	9	3	190	13,2	0,65	22,1
Pp	1501-5	10	3	160	12,5	0,7	18,3
Pp	1501-5	11	3	28	13	0,6	3,6
Pp	1501-5	12	3	3	12	0,55	0,5
Pp	1501-5	13	3	10	14,6	0,8	0,9
Pp	1501-5	14	3	105	13	0,6	13,5

Table 50. Phenotypic results of inoculation with the pathogen *Pp* for some progeny of 1501-5 and the controls SusPtrit (SusP), Vada and L94. These individuals were inoculated separately and are therefore displayed separately. Width, length and the amount of pustules is given for each individual. Colors indicate highest and lowest value, from dark green (lowest) to dark red (highest). Plant 26 died and was therefore not phenotyped.

Pathogen	Genotype	Plant number	Pustules	Length	Width	IF	RIF
Pp	Vada	1	1	10.6	0.8	0.1	1.2
Pp	Vada	2	5	11.7	0.8	0.5	5.6
Pp	Vada	3	10	11.7	0.8	1.1	11.2
Pp	Vada	4	0	7.5	0.6	0.0	0.0
Pp	L94	1	0	5.5	0.8	0.0	0.0
Pp	L94	2	1	15.6	0.8	0.1	0.8
Pp	L94	3	0	11	0.7	0.0	0.0
Pp	L94	4	0	14.5	0.9	0.0	0.0
Pp	SusP	1	129	17	0.8	9.5	99.2
Pp	SusP	2	135	15	0.85	10.6	110.7
Pp	SusP	3	84	13	0.75	8.6	90.1
Pp	1501-5	15	3	10.7	0.45	0.6	6.5
Pp	1501-5	16	18	17.3	0.7	1.5	15.5
Pp	1501-5	17	8	13.1	0.7	0.9	9.1
Pp	1501-5	18	17	10.4	0.6	2.7	28.5
Pp	1501-5	19	33	14.4	0.7	3.3	34.2
Pp	1501-5	20	12	11.6	0.6	1.7	18.0
Pp	1501-5	21	5	14	0.7	0.5	5.3
Pp	1501-5	22	29	11.6	0.6	4.2	43.6
Pp	1501-5	23	44	12.4	0.6	5.9	61.8
Pp	1501-5	24	16	11.6	0.6	2.3	24.0
Pp	1501-5	25	9	7	0.4	3.2	33.6
Pp	1501-5	26	x	x	x		
Pp	1501-5	27	6	11.6	0.6	0.9	9.0
Pp	1501-5	28	11	10	0.6	1.8	19.2
Pp	1501-5	29	14	13.4	0.7	1.5	15.6
Pp	1501-5	30	22	8.8	0.5	5.0	52.3

Table 51. Phenotypic results of inoculation with the pathogen *Phb_Isr* for the progeny of 1501-2 to 1501-5 and the controls SusPtrit (SusP) and L94. Width, length, amount of pustules and the box number is given for each individual. Colors indicate highest and lowest value, from dark green (lowest) to dark red (highest

Pathogen	Genotype	Plant number	Box	Pustules	Length	Width	IF
Phb_Isr	L94		1	0	0	0	0,0
Phb_Isr	L94		1	0	0	0	0,0
Phb_Isr	L94		1	3	15,5	0,9	0,2
Phb_Isr	L94		1	3	13,5	0,85	0,3
Phb_Isr	L94		2	5	15,3	0,9	0,4
Phb_Isr	L94		2	9	16,5	0,9	0,6
Phb_Isr	L94		2	2	18	1	0,1
Phb_Isr	L94		2	0	18	0,9	0,0
Phb_Isr	SusP		1	44	8,5	0,7	7,4
Phb_Isr	SusP		1	160	14,8	0,85	12,7
Phb_Isr	SusP		1	54	11,2	0,8	6,0
Phb_Isr	SusP		2	125	15,2	0,8	10,3
Phb_Isr	SusP		2	150	16	0,8	11,7
Phb_Isr	SusP		2	50	6	0,75	11,1
Phb_Isr	SusP		2	140	14	0,75	13,3
Phb_Isr	SusP		2	110	16,3	0,9	7,5
Phb_Isr	1501-5	1	2	10	15,5	0,8	0,8
Phb_Isr	1501-5	2	2	0	14	0,75	0,0
Phb_Isr	1501-5	3	2	2	17	0,8	0,1
Phb_Isr	1501-5	4	2	25	15	0,7	2,4
Phb_Isr	1501-5	5	2	17	15,5	0,55	2,0
Phb_Isr	1501-5	6	2	54	14,2	0,75	5,1
Phb_Isr	1501-5	7	2	7	17,2	0,9	0,5
Phb_Isr	1501-5	8	2	38	16	0,8	3,0
Phb_Isr	1501-5	9	2	48	14,2	0,75	4,5
Phb_Isr	1501-5	10	2	8	13,5	0,75	0,8
Phb_Isr	1501-5	11	2	2	15,2	0,65	0,2
Phb_Isr	1501-5	12	2	60	13,2	0,7	6,5
Phb_Isr	1501-5	13	2	48	16,4	0,85	3,4
Phb_Isr	1501-5	14	2	90	15,7	0,9	6,4
Phb_Isr	1501-5	15	2	140	15,2	0,8	11,5
Phb_Isr	1501-5	16	2	12	17	0,8	0,9
Phb_Isr	1501-5	17	2	9	15	0,75	0,8
Phb_Isr	1501-5	18	2	18	18	0,9	1,1
Phb_Isr	1501-5	19	2	1	17,3	0,8	0,1
Phb_Isr	1501-5	20	2	2	19	0,8	0,1
Phb_Isr	1501-5	21	2	85	15	0,9	6,3
Phb_Isr	1501-5	22	2	20	15,5	0,75	1,7
Phb_Isr	1501-5	23	2	100	16,2	0,85	7,3
Phb_Isr	1501-5	24	2	26	16,3	0,7	2,3
Phb_Isr	1501-5	25	2	30	16	0,8	2,3

Phb_Isr	1501-5	26	2	28	16,5	0,8	2,1
Phb_Isr	1501-5	27	2	70	16,5	0,85	5,0
Phb_Isr	1501-5	28	2	80	14	0,8	7,1
Phb_Isr	1501-5	29	2	11	15	0,65	1,1
Phb_Isr	1501-5	30	2	10	16,8	0,7	0,9
Phb_Isr	1501-5	31	2	23	15	0,8	1,9
Phb_Isr	1501-5	32	2	110	14,2	0,9	8,6
Phb_Isr	1501-5	33	2	8	14,1	0,7	0,8
Phb_Isr	1501-5	34	2	1	14	0,65	0,1
Phb_Isr	1501-5	35	2	75	15	0,8	6,3
Phb_Isr	1501-2	1	1	15	15	0,75	1,3
Phb_Isr	1501-2	2	1	10	15	0,8	0,8
Phb_Isr	1501-2	3	1	9	16,2	0,8	0,7
Phb_Isr	1501-2	4	1	15	15,2	0,75	1,3
Phb_Isr	1501-2	5	1	71	16,5	0,8	5,4
Phb_Isr	1501-2	6	1	14	14,3	0,8	1,2
Phb_Isr	1501-2	7	1	13	11	0,65	1,8
Phb_Isr	1501-2	8	1	9	16,8	0,8	0,7
Phb_Isr	1501-2	9	1	40	14	0,8	3,6
Phb_Isr	1501-2	10	1	25	14	0,75	2,4
Phb_Isr	1501-2	11	1	2	17	0,9	0,1
Phb_Isr	1501-2	12	1	61	15	0,8	5,1
Phb_Isr	1501-2	13	1	0	0	0	0,0
Phb_Isr	1501-2	14	1	0	0	0	0,0
Phb_Isr	1501-2	15	1	14	15	0,8	1,2
Phb_Isr	1501-2	16	1	22	17,5	0,8	1,6
Phb_Isr	1501-2	17	1	49	16	0,8	3,8
Phb_Isr	1501-2	18	1	3	15	0,8	0,3
Phb_Isr	1501-2	19	1	4	16,6	0,9	0,3
Phb_Isr	1501-2	20	1	10	16	0,75	0,8
Phb_Isr	1501-2	21	1	6	17,5	0,85	0,4
Phb_Isr	1501-2	22	1	44	13,5	0,7	4,7
Phb_Isr	1501-2	23	1	8	16	0,8	0,6
Phb_Isr	1501-2	24	1	16	16,5	0,85	1,1
Phb_Isr	1501-2	25	1	18	15,5	0,8	1,5
Phb_Isr	1501-2	26	1	3	18,5	0,9	0,2
Phb_Isr	1501-2	27	1	24	16,6	0,8	1,8
Phb_Isr	1501-2	28	1	24	16	0,85	1,8
Phb_Isr	1501-2	29	1	6	16,5	0,85	0,4
Phb_Isr	1501-2	30	1	9	12	0,8	0,9
Phb_Isr	1501-2	31	1	14	15,4	0,8	1,1
Phb_Isr	1501-2	32	1	3	16,3	0,7	0,3
Phb_Isr	1501-2	33	1	18	16,5	0,8	1,4
Phb_Isr	1501-2	34	1	7	15,5	0,75	0,6