



THE ASSOCIATION BETWEEN HOST AND NON-HOST BASAL RESISTANCE

BSc. thesis research

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SUMMARY

Quantitative resistance is due to genes with relatively small, quantitative effects, located on so called quantitative trait loci (QTL). Quantitative resistance does not provide complete protection. But compared with qualitative resistance, it is believed to be more durable and effective even after a period of widespread agricultural use. Basal resistance is regarded as quantitative resistance. The knowledge exploited on the genetic characterization and function of basal resistance genes, will help breeding for durable resistance in barley and other crops.

Many studies on host and non-host basal resistance in barley previously provided clues of the possible association between the host basal resistance (partial resistance) and non-host basal resistance (non-host resistance). In this thesis, three different studies were executed to contribute important information to one major research of the possible association between the host and non-host basal resistance.

The first study is fine mapping of *Rphq11* and *Rphq16*, two QTLs for partial resistance of barley to barley leaf rust in a “fast and dirty” way by using homologous recombinant lines for genotyping and disease test. *Rphq11* was fine mapped into two genetic windows. One is between WBE144 and K14 (0.1 cM). The other genetic window flanked by GBMS244 and WBE130 (0.4 cM). Based on the synteny with rice, the physical size of genetic windows of *Rphq11* is about 22kb and 43kb, respectively. The genetic window of *Rphq16* was narrowed down by 7.4 cM, in the interval between ABC11948_3 and TC181991_2 (3.1 cM). the physical size of genetic window of *Rphq16* is approximately 175kb. . Identification of target basal resistance genes could help with developing molecular markers for breeding programs in the future study, and verify responsible genes quickly.

In the second study, marker development for fine mapping of *Rnhq* was executed by using premature genome sequencing project of barley using 454 sequencing technology. One SCAR marker (HVVMRXALLeA0361C16b) was found, but it cannot be used for fine mapping of *Rnhq* because it was mapped outside of *Rnhq* region. CAPs marker was tried to be developed from SNP found in HVVMRXALLeA0313H07_3. However, it did not show polymorphism after digestion, hence no CAPs marker was obtained. No useful molecular markers were obtained after testing 33 primer pairs.

The third study intended to test the specificity of partial resistance and non-host resistance QTLs towards homologous and heterologous rusts. Near isogenic lines (NILs) of targeted QTLs were generated. The result illustrated that partial resistance QTLs, *Rphq2*, *Rphq3*, *Rphq11* and *Rphq16* and the non-host resistance QTL, *Rnhq*, in barley, have an effects on both partial resistance towards homologous leaf rust, as well as non-host resistance towards heterologous leaf rusts, *P. hordei-murini* and *P. hordei-secalini*. Additionally, a significant positive association was found between parameter of relative latent period in seedling stage and proportion of early abortion at infection sites, which indicated that there is a possible association between partial resistance QTLs and non-host resistance QTLs. Study on effect of basal resistance can help to accumulate the effective QTLs into cultivars of interest to create an artificial (near) non-host cultivars.

Chapter 1 Introduction

In crop cultivation, potential crop yields can hardly be reached due to different stress factors. It is the goal of plant breeders to improve the crops' ability to cope with all the stress factors (Niks, Lindhout, 2006). The stress factors can be divided into biotic and abiotic.

Pathogens, disease causal agent, can be categorized into biotic factors. The word "disease" refers to a physiological disturbance in the whole plant, leading to symptoms such as yellowing, wilting, stunting and mal-formation. Such symptoms are the most notable indicators of infections by viruses, phytoplasmas, bacteria and vascular wilt fungi, etc. (Niks, Lindhout, 2006).

Cultivated barley (*Hordeum vulgare* L.) belongs to the family Poaceae, in the Triticeae tribe (Backes *et al.* undated). There are winter and summer annual forms of barley. Based on inflorescence, barley can be divided into two-rowed barley and six rowed barley. Cultivated barley is an autogamous diploid plant ($2n = 2x = 14$), but tetraploids ($2n = 4x = 28$) and hexaploids ($2n = 4x = 42$) can also be found. The seven chromosomes of barley were named 7H, 2H, 3H, 4H, 1H, 6H and 5H, respectively, because of their homology to the seven chromosomes of wheat (Franckowiak *et al.*, 1997).

Leaf rust (*Puccinia hordei* Oth) is one of the important worldwide fungal diseases of barley, which can cause severe yield losses. Ochoa and Parlevliet (2007) stated that there is a high correlation between yield loss and severity of leaf rust infection. The yield response associated with the disease intensity, the time of onset of the disease relative to host development, and the duration of the epidemic (Lim and Gaunt, 1986; Whelan, *et al.*, 1997). Reported yield loss ranges between 30% - 60% depending on the barley cultivar used (Cotterill, *et al.*, 1992; Teng, 1978 cited by Das *et al.*, 2007; Ochoa and Parlevliet, 2007). In order to reduce such high yield losses, developing resistant cultivars is necessary. It is also for economic survival of farmers and help to reduce the harmful effects of pesticide and fungicide on environment and consumer (Williams, 2003).

When referring to plant pathosystem, a four phased zigzag model, presented by Jones and Dangl (2006), nicely illustrated the interaction between the plant and the pathogen (Figure 1.1). In the first phase, the pathogen associated molecular pattern (PAMP) is recognized by the plant through pattern recognition receptors (PRRs). And the detection elicits PAMP-triggered immunity (PTI) or nonspecific basal resistance. Continuing the plant-pathogen arms race (Phase 2), successful pathogens will circumvent PTI. In a plant with resistance gene(s) (R gene), the pathogen which successfully evade PTI will be recognized by R gene and trigger effector-triggered immunity (ETI) (third phase). ETI is often regarded as an amplified version of PTI, leading to hypersensitive cell death (HR). Under selection pressure, the pathogen avoids ETI either through discarding or diversifying their effectors. Alternatively, pathogens may acquire additional effectors that suppress ETI. In the fourth phase of plant-pathogen arms race the plant will gain new or diversified R genes.

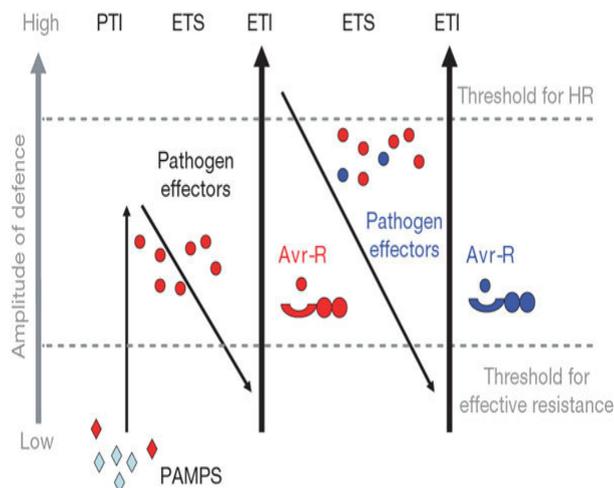


Figure 1.1 Four phased zigzag model of plant- pathogen interaction (Jones and Dangl, 2006)

Qualitative resistance with hypersensitivity has been extensively studied in plant-pathogen systems. This resistance can be explained with the gene-for-gene model between resistance genes in the host and avirulence genes in the pathogen (Flor 1971). It is widely used in plant breeding programs, but qualitative resistance is often not durable because the resistance genes can easily be overcome by new variants of the pathogen. Compared with qualitative resistance, quantitative resistance does not provide complete protection, but it is believed to be more durable and effective even after a period of widespread of agricultural use (Clofford, 1985).

The first phase of the illustration in Figure 1.1 is nonspecific basal resistance, which can be subdivided into host basal resistance and non-host basal resistance. Both are quantitative resistance regulated by many genes (Parlevliet and Van Ommeren, 1975; Jafary *et al.*, 2006 and 2008).

Host basal resistance also named as partial resistance, is a polygenically inherited host resistance that retards epidemic development in the field, although plants show a high, compatible, infection type (Parlevliet and Van Ommeren, 1975; Parlevliet, 1976; Parlevliet, 1978). Barley with high level of partial resistance allows a lower infection frequency of the adapted barley leaf rust fungus *P. hordei*. The pathogen also has longer latent period and lower sporulation rate (Parlevliet, 1979).

Non-host basal resistance, also called non-host resistance, is the resistance observed in non-host plant species, which the entire species is resistant to a specific pathogen. The specific pathogen is inappropriate or unadapted (hereafter called heterologous pathogen). The non-host status of barley to rust fungal species is not a black-and-white division, but intermediate “near non-host” status occurred as well (Atienza *et al.* 2004). This character of barley enables it to be a suitable model to be investigated on genetics and mechanism of partial resistance and non-host resistance.

Numerous QTLs for barley partial resistance and non-host resistance have been mapped (Qi *et al.*, 1998; Jafary *et al.*, 2006; Marcel *et al.*, 2007). The genetic segregation for resistance to rusts to which barley has a near non-host status tends to be associated with segregation for levels of quantitative basal resistance to *P. hordei* in barley. This indicates that the genes for partial resistance seem to play similar roles as those genes governing non-host resistance in basal resistance (Zhang *et al.*, 1994; Hoogkamp *et al.*, 1998; Jafary *et al.*, 2006).

Other evidence of association is demonstrated by Niks *et al.* (1983, 1986, and 1989) who reported that mechanism of partial resistance is prehaustorial similar to the mechanism of non-host resistance of barley to heterologous rust fungi based on the histological studies. This suggests that the two resistance types are based on shared principles. Partial resistance resembles a weak form of non-host resistance

Considering the emerging evidence of association between partial resistance and non-host resistance, the current project exploits the knowledge on barley partial resistance and availability of strategic plant material to study barley non-host resistance. The knowledge exploited on this theme will help plant breeding programs to find important applications and improve disease resistance of barley cultivar and other crop species. On the one hand, the identification of genes involved in basal resistance will enable molecular markers development, which could be utilized in breeding program or verify responsible genes quickly. On the other hand, study on function of basal resistance can help to accumulate the effective QTLs into host cultivars, which could be turned into artificial (near) non-host, providing high level of durable resistance against specific pathogen. Durable resistant cultivars are developed for preventing extensively use of fungicides and pesticide which are harmful for the environment, as well as for economical survival of growers.

This thesis reports three different studies which together contribute important information to one major research on the association between host and non-host basal resistance.

The first study is Fine mapping of *Rphq11* and *Rphq16*, QTLs for partial resistance to barley leaf rust (Chapter 2). The ultimate objective is to clone the gene(s) responsible for partial resistance by using map based cloning approach. The first step in the approach is the mapping of the QTLs, which was done by Thierry (2007). The second step is fine mapping of the QTLs of interests, which is one of the main objectives in this thesis.

The second one intended to generate more molecular markers needed for fine mapping of *Rnhq*, a non-host resistance QTL in barley (Chapter 3). The BAC end sequences of BAC clones spanning *Rnhq* were exploited to achieve this objective.

The third study is to test the effect of partial resistance and non-host resistance QTLs towards homologous and heterologous rusts (Chapter 4). Four partial resistance QTLs and one non-host resistance QTL of our interest were now available in near isogenic lines (NIL). The result may give indication on possible association between non-host resistance and partial resistance in barley towards leaf rusts.

Research Objective and Question

Main objective: To study the association between partial resistance and non-host resistance of barley towards leaf rust.

Main research question: Is there any possible association between partial resistance and non-host resistance of barley towards leaf rust?

Sub Objective 1: Fine mapping of *Rphq11* and *Rphq16*, QTLs for partial resistance to barley leaf rust.

Research question 1: what is the precise genetic window of *Rphq11* and *Rphq16* and what are the candidate genes present in the region?

Sub Objective 2: Marker development for *Rnhq*, a non-host resistance QTL in barley by using the BAC end sequences. (Chapter 3)

Research question 2: what is the precise genetic window of *Rnhq* and what are the candidate genes present in the region?

Sub Objective 3: To test the specificity of the effect of partial resistance and non-host resistance QTLs towards homologous and heterologous rusts.

Research question 3: Is there any specificity of effect on partial resistance and non-host resistance QTLs towards homologous and heterologous rusts?

Report structure

This thesis report contained five chapters. The first is the introduction, which described barley, leaf rust, plant pathosystem, and the reason why to study the association between partial resistance and non-host resistance of barley towards leaf rust. Three different experiments under the theme were also briefly introduced. Chapter 2, 3 and 4 detailed revealed each sub objective in introduction, materials and methods, result, discussion and conclusion. Finally, Chapter 5 is the general conclusion for the thesis study.

CHAPTER 2 FINE MAPPING OF *RPHQ11* AND *RPHQ16*, QTLs FOR PARTIAL RESISTANCE TO BARLEY LEAF RUST

2.0 INTRODUCTION

There are many agriculturally important traits such as yield, quality and some forms of disease resistance are regulated by many genes, naming as “quantitative traits” (Collard *et al.* 2005). The gene regions within a genome associated with a specific trait is regarded as “quantitative trait loci” (QTL). “QTL mapping” is a process to identify genomic regions linked with traits (Collard *et al.* 2005). It depends on the principle that genes and markers segregate via chromosome recombination during sexual reproduction, hence making it possible to analyze in the progeny (Paterson, 1996). After identifying the QTLs for trait of interest, near isogenic lines (NILs) development and “fine mapping”(also known as “high-resolution mapping”) need to be performed in order to allow the study and cloning of one or more specific QTLs,

Rphq11 and *Rphq16* both are partial resistance QTLs against *P. hodei* 1.2.1 targeted for NILs development. They are QTLs effective at seedling stage only (Kuijken, 2009; Bouchon, 2009). *Rphq11* and *Rphq16* were mapped in Steptoe x Morex and OWB barley mapping populations, respectively (Marcel, 2007). *Rphq11*, an allele from Steptoe on chromosome 2H and *Rphq16*, an allele from Dom on chromosome 5H were the QTL with the highest effect on resistance, explaining 34% and 33%, respectively, of the variation.

Fine mapping can be achieved by using NILs (Blair *et al.*, 2003). The NILs development program was initiated by crossing Steptoe and Dom, donor plant for each QTL, respectively, to SusPtrit. SusPtrit is an accession developed with hyper-susceptible to several heterologous rusts and homologous rust in order to study barley partial resistance and non-host resistance (Atienza, 2004). Both Steptoe and Dom also contain another partial resistance QTL, *Rphq15* and *Rphq17* respectively. During NILs development, marker assisted selection was against *Rphq15* and *Rphq17*. The marker assisted selection was performed using two or more flanking markers recommended by Visscher *et al.* (1996).

The approach of using NILs for fine mapping is laborious and time consuming. Hence, in parallel with the NILs development of *Rphq11* and *Rphq16*, fine mapping was carried out (Figure 2.1). This is a “fast and dirty” approach with the intension to accelerate the fine mapping process. At generation BC₁, plant materials having only *Rphq11* and *Rphq16*, respectively, were found (Lorriaux, 2007; Yeo, 2007), and the effect of each QTL was clearly observed. It was the first attempt when fine-mapping was performed on these materials. NILs development and fine-mapping were split into independent work at generation BC₁ of the QTLs respectively, but is executed in parallel.

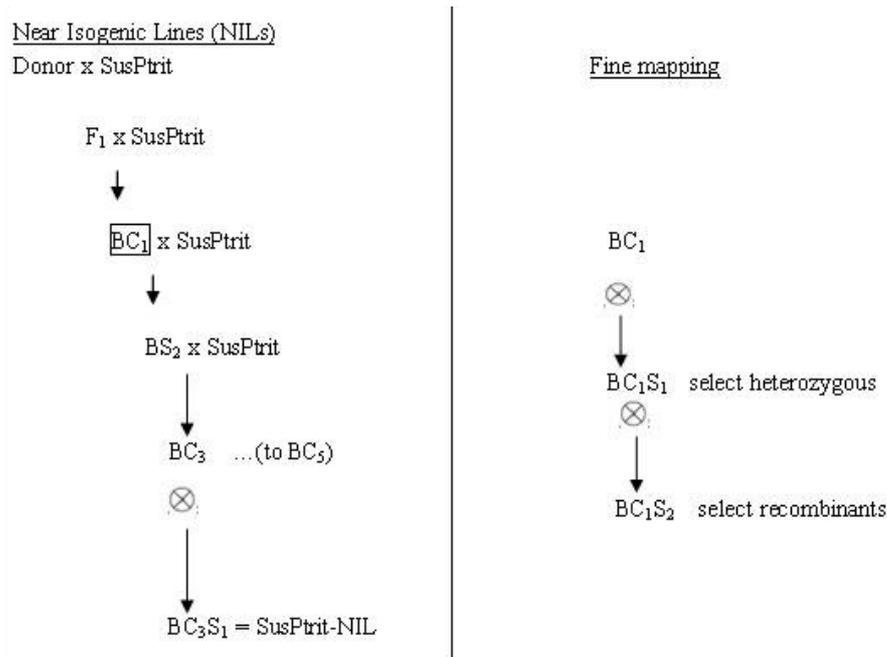


Figure 2.1 Flowchart of NILs development and fine mapping of *Rphq11* and *Rphq16*.

The latest fine mapping result of *Rphq11* and *Rphq16* was based on strategic heterozygous recombinant lines. The two QTLs were fine-mapped into a genetic window of 0.82 cM and 0.7 cM, respectively (Kuijken, 2009; Bouchon, 2009). In order to further fine map *Rphq11* and *Rphq16*, and give a better statistical representation, more molecular markers were made available for *Rphq11* and *Rphq16*, and homozygous recombinant lines were selected and used in this study to fine map *Rphq11* and *Rphq16*. Homozygous recombinant lines of *Rphq11* were F₆ plants, selected from F₅ heterozygous recombinant lines derived from Steptoe x SusPtrit. Whereas for *Rphq16*, the plant materials are F₄ plants and derived from Dom x SusPtrit.

2.1 MATERIALS AND METHODS

Strategic homozygous recombinant lines representing different recombination points were used to fine map *Rphq11* and *Rphq16*. A total of 34 homozygous recombinant lines were used for *Rphq11* and 32 lines for *Rphq16*. Parental lines such as Vada, L94, Steptoe, Morex, Dom, Rec, and QTL-NILs of *Rphq11* and *Rphq16* (*Sus-QTL11-F2-BC₅S₁* and *Sus-QTL16-BC₆S₁*, respectively) were included as references. Three replications were performed. In each replication, recombinant lines were arranged into 4 trays for *Rphq11* and 5 trays for *Rphq16*, and reference lines were sowed in all trays.

The homozygous recombinant lines together with the reference lines were challenged with *P. hordei* isolate 1.2.1., an adapted leaf rust species. Ten to twelve days after sowing, the first leaf of all the lines were fixed on the tray, with adaxial side up. Three mg of spores were used for each tray, resulting in around 180 urediospores per cm² (Qi *et al.*, 1998). The inoculums were diluted 10 times with lycopodium spores before

dusted over the tray using inoculation tower. The dusted tray was placed in humidity chamber to incubate the spores overnight (around 8 hours) at 100% relative humidity at 17°C to 18°C. After incubation, the seedlings were transferred to a greenhouse compartment where the temperature was set at $14 \pm 3^\circ\text{C}$ with 30-70% relative humidity.

Latent Period (LP50S) was scored for this experiment. LP50S is an important parameter. It is the period of time needed for fifty percent of the total pustules becoming mature in seedling plants. It can be calculated with the following formula:

$$\text{LP50S} = T1 + (T2 - T1) \times (N100/2 - N1) / (N2 - N1)$$

T1 = the time point just before 50% of the pustules are mature

T2 = the time point just after 50% of the pustules are mature

N1 = number of mature pustules at T1

N2 = number of mature pustules at T2

N100/2 = half of the total mature pustules number

To determine whether there was genotypic effect on RLP50S (LP50S relative to SusPtrit) within selected homozygous recombinants, one way ANOVA design was performed in SPSS. Lines were grouped into AA and BB according to their markers' genotype in the genetic distance between the flanking markers of respective QTLs. A genetic effect was considered to be present in a family at a significance level of $P=0.05$.

2.2 RESULT

2.2.1 FINE MAPPING OF RPHQ11

The identified homozygous recombinants using flanking markers were already genotype previously with existing and newly developed markers around *Rphq11*. Based on the genotypic data together with the obtained phenotypic data, *Rphq11* is split into two genetic windows flanked by WBE144 and K14 (0.1 cM), and GBS244 and WBE130 (0.4 cM), respectively (Figure 2.2). According to the analysis of one way ANOVA using SPSS, plants with genotype AA, allele of SusPtrit, in both possible genetic regions of QTLs presented significant lower LP50S relative to SusPtrit (RLP50S), than lines with genotype of BB, which is allele of Steptoe, the donor plant (P values are 0.025 and 0.033 for the two regions respectively).

2.2.2 FINE MAPPING OF RPHQ16

Similar to *Rphq11*, the homozygous recombinant lines selected using the flanking marker were genotyped previously using existing and newly developed molecular markers around *Rphq16*. Thirty-two homozygous recombinant lines representing different recombination points in the region of *Rphq16* were used for this experiment. The phenotypic data obtained in this study together with two additional replications from another MSc student (Dido, 2010) were used for the analysis in this study. Three lines (DOM147.24, DOM1.3 and DOM29.13) were excluded during analysis because of lack of phenotypic data.

The result can be concluded that the genetic window of *Rphq16* is now 3.1 cM between the flanking markers of ABC1948_3 and TC181991_2 (Figure 2.3), although there are three lines (DOM128.13, DOM111.3 and DOM201.7) showing conflicting phenotypic data.

One way ANOVA in SPSS was used to test the significant difference between plants with genotype AA (allele of SusPtrit) and BB (allele of DOM) in the possible genetic region of QTL. There is a phenotypic effect of partial resistance on the recombinant lines. Plants with DOM allele in the identified QTL region showed significantly higher value of RLP50S (P= 0.01).

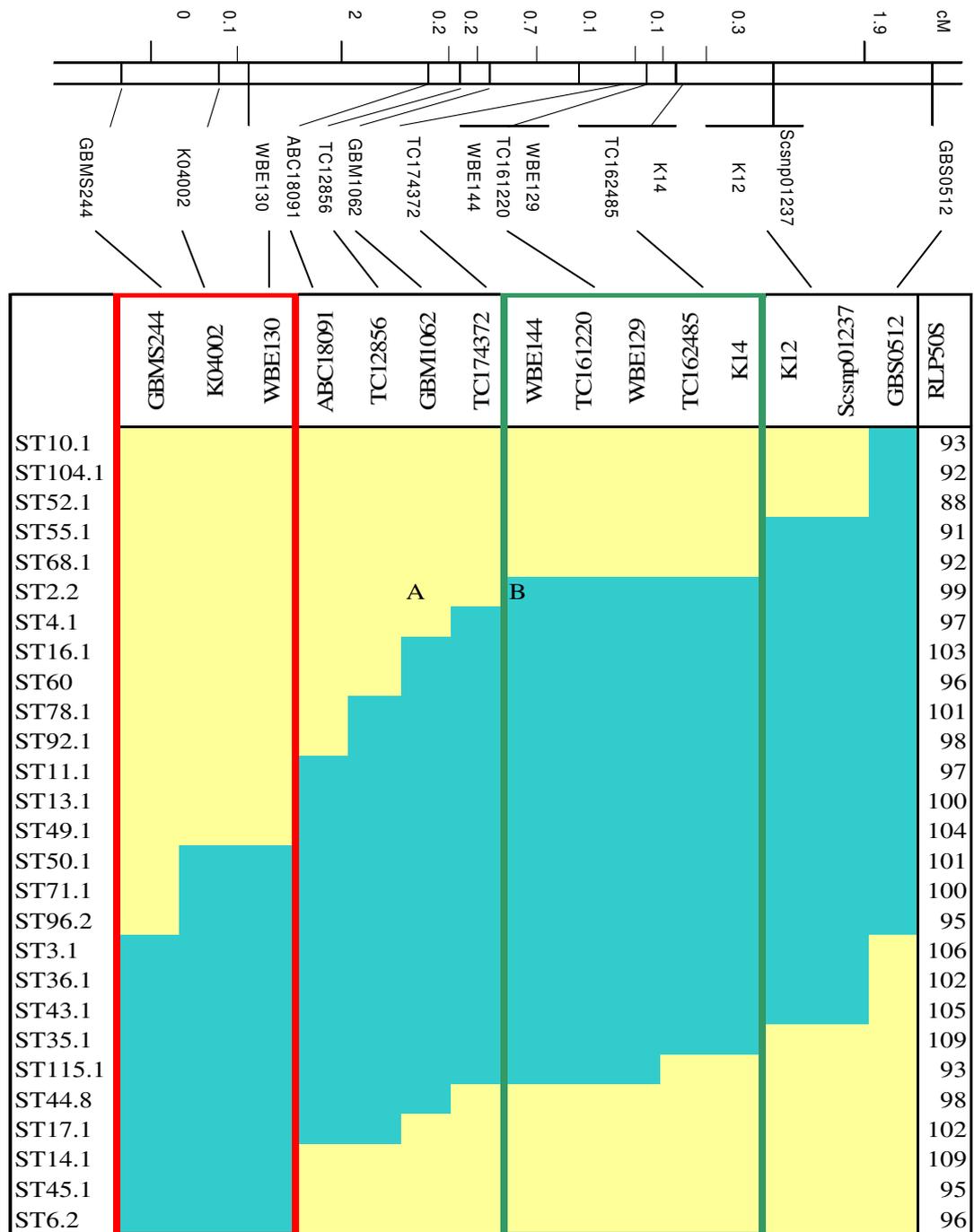


Figure 2.2 Genotype and phenotype of *Rphq11* homozygous recombinant lines (Yellow area means SusPtrit introgression; blue area means Steptoe introgression area; “A” represents homozygous SusPtrit allele; “B” represents homozygous Steptoe allele; regions boxed with red and green lines are the possible QTL regions.)

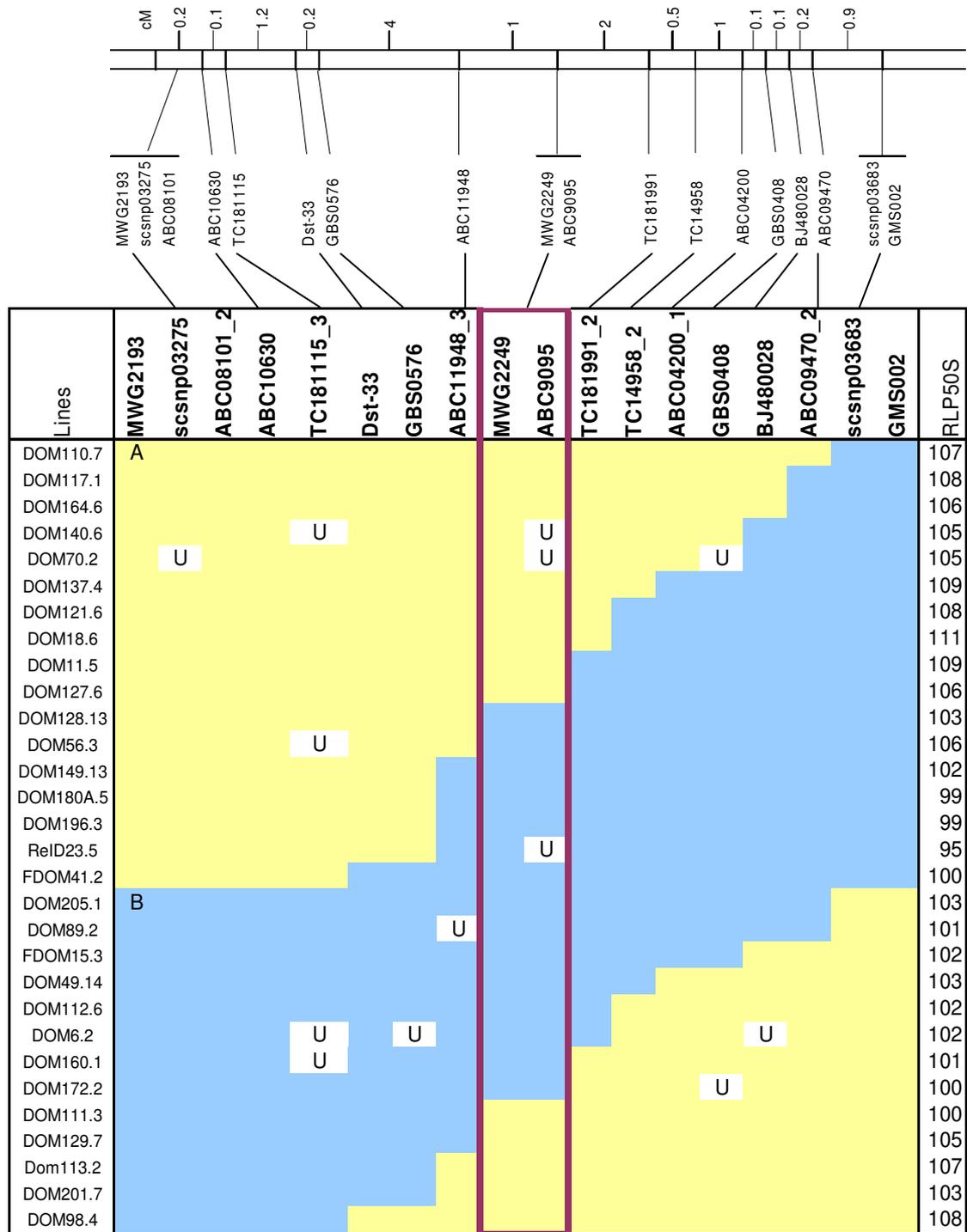


Figure 2.3 Genotype and phenotype of *Rphq16* homozygous recombinant lines selected for this study (green area means *SusPtrit* introgression; grey area means *Steptoe* introgression; “A” represents homozygous *SusPtrit* allele; “B” represents homozygous *Dom* allele; “U” means unknown; lines with red are conflicting in phenotypic and genotypic data; regions boxed with purple line are the QTL regions predicted.)

2.3 DISCUSSION

2.3.1 FINE MAPPING OF *RPHQ11*

This fast and dirty approach speeds the fine mapping process, and it was proven applicable to fine map *Rphq11* and *Rphq16*. Previously, *Rphq11* was positioned in a 0.82 cM genetic interval between the markers K14 (Uni19962) and GBM1062. In this study, based on three replicates of disease test, *Rphq11* is split into two genetic windows. One genetic window was flanked by WBE144 and K14 (0.1 cM). Another genetic window is between GBMS244 and WBE130 (0.4 cM). In both regions there are significant differences (P-values are 0.025 and 0.033 respectively) between phenotypic data from two different kinds of genotypes (A and B). It indicates that the partial resistance QTL in these two regions is effective.

The lines (e.g. ST96.2) having the two newly identified QTLs in *Rphq11* region did not show longer latent period compared to those lines with only one of the regions having the Steptoe allele. It seems like the two QTLs do not have additional effect. Maybe one of them is switched off when the two of them are present. However, more strategic recombinants targeting the two newly identified QTLs is needed to verify and clarify the result.

Based on the synteny with rice, the physical sizes of the two genetic windows of *Rphq11* are approximately 22kb and 43kb, respectively. The candidate genes found the genetic window flanked by WBE144 and K14 are glutathione peroxidase and serine racemase. In the other genetic window flanked by GBMS244 and WBE130, protein kinase and SHR5-receptor-like kinase were found.

2.3.2 FINE MAPPING OF *RPHQ16*

Based on 5 replications, *Rphq16* is narrowed down into a region of 3.1 cM between ABC11948_3 and TC181991_2. This is in agreement with previous reported position, between MWG2249 and ABC09095 (0.7 cM). It seems like the present result did not further fine map the QTL. However this was due to the position of MWG2249 and ABC09095. Previous genetic map had the position of these two markers resolved. The current new genetic map with improved resolution did not resolve the position of MWG2249 and ABC09095 because no recombination was found between these two markers. This may due to missing data resulted from the poor performance of ABC09095. Assuming these two markers were not resolved in previous genetic map, *Rphq16* would be positioned in a genetic window of 10.5 cM flanked by GBS0576 and GBS0408. In comparison with the present result, the genetic window is reduced by 7.4 cM. In the future study, in orders to deeply study *Rphq16*, more molecular markers are needed in the region of 3.1 cM between MWG2249 and ABC09095.

The physical distance of *Rphq16* genetic window is 175kb based on synteny with rice. The candidate genes found in the genetic window are oxidoreductase and glutathione S-transferase.

2.4 CONCLUSION

Fine mapping of *Rphq11* and *Rphq16*, QTLs for partial resistance (host basal resistance) to barley leaf rust was executed in a “fast and dirty” way by using homozygous recombinant lines. *Rphq11* was split into two genetic windows. One is between WBE144 and K14 (0.1 cM), and its candidate genes are glutathione peroxidase and serine racemase. The other genetic window flanked by GBMS244 and WBE130 (0.4 cM), protein kinase and SHR5-receptor-like kinase were found as its candidate genes.

The genetic window of *Rphq16* was narrowed down by 7.4 cM, in the region between ABC11948_3 and TC181991_2 (3.1 cM). The candidate genes found in the genetic window are oxidoreductase and glutathione S-transferase.

Based on the synteny with rice, , the physical sizes of the two genetic windows of *Rphq11* are approximately 22kb and 43kb. For *Rphq16*, it is approximately 175kb.

The identification of candidate genes involved in basal resistance will not only enable molecular markers development in the future studies, but also to verify responsible genes explaining the QTL quickly.

CHAPTER 3 MARKER DEVELOPMENT FOR *RNHQ*, A NONHOST RESISTANCE QTL IN BARLEY

3.0 INTRODUCTION

The use of molecular markers to enhance plant breeding efforts is being widely studied (Dudley, 1992). Molecular markers can be used to identify and manipulate chromosome segments QTL controlling quantitative traits. In this study, molecular markers are needed to improve the resolution of genetic map for *Rnhq*.

Rnhq is a QTL for non-host resistance. It was mapped in L94 x Vada recombinant inbred line (RIL) mapping population. The population was challenged with the heterologous rusts *P. triticina* and *P. hordei murini* at the seedling stage (Niks, Fernandez *et al.* 2000). Samples were evaluated macroscopic and microscopically, and a QTL from Vada was discovered, which was effective against *P. hordei murini* and to a lesser extent to *P. triticina*. This non-host QTL (*Rnhq*) was mapped on the long arm of chromosome 1 (7H) and seemed to have no effect on the host pathogen *P. hordei*. Work on *Rnhq* was continued and a near isogenic line (NIL) with an L94 background was created (Dijk, 2007). Fine mapping of *Rnhq* was started by Jafary (2006), using the resistant NIL *L94-Rnhq* and the susceptible L94 as parents. After that Dijk (2007) narrowed down the genetic window into 0.78 cM between the GBM1359 and SKT1 markers. More recently, NILs with the SusPtrit background was made available. It suits more for non-host resistance study because of its unique susceptibility to heterologous rusts (Atienza *et al.* 2004).

The peak marker for *Rnhq* is SKT1. It is our interest to fine map *Rnhq*. To date, not many PCR based markers are saturated around this region. Hence, more molecular markers around *Rnhq* are desirable. This chapter makes use of a premature genome sequencing project of barley which uses 454 sequencing technology.

3.1 MATERIALS AND METHODS

In this study, we made use of the information generated from a premature genome sequencing project of barley using 454 sequencing technology. Based on the peak markers of *Rnhq*, three unique contigs of BAC clones spanning over *Rnhq* were identified. In these contigs, there are nine BAC clones (18 BAC ends) selected to exploit their BAC end sequences. The primers were designed by using the software DNASTAR®. The PCR product size was designed to range from 700-800 bp depends on the sequence entered and the primer length ranges from 20 to 24 bp. Gradient-PCR was performed for each pair of designed primers in order to determine the optimal annealing temperature (Appendix 1). Using optimized annealing temperature, PCR was performed on L94, Vada and SusPtrit. SCAR markers were obtained by length polymorphism or allele-specific amplification directly after PCR. When, primer pairs amplified DNA sequences of the same size in parental lines, the PCR products were sent for direct sequencing. The sequence information was searched for presence of single nucleotide polymorphisms (SNPs), which can be exploited for CAPS or derived-CAPS (dCAPS) markers development. Suitable restriction enzymes can be found with the dCAPS finder program (<http://helix.wustl.edu/dcaps/dcaps.html>). Once the appropriate restriction enzymes had been identified, they were tested. If polymorphism is observed between L94, Vada and SusPtrit, CAPS markers are developed. Newly developed markers were mapped using the software RECORD (van Os *et al.* 2005).

3.2 RESULT

In total there were 33 pairs of primers were designed. Seventeen pairs of them were discarded due to poor amplification and 16 pairs were processed further. One SCAR dominant marker (HVVMRXALLeA0361C16b) was found. It shows polymorphism between L94/SusPtrit and L94/Vada. The SCAR marker was mapped using L94 x Vada mapping population. Based on RECORD, the position of this marker is in chromosome 3H between E35M48-410 and E33M61-131 a region of 5.7 cM in barley integrated map. It is not in the region of *Rnhq*.

The other 15 primer pairs with good amplification but without polymorphism were sent for sequencing. The sequence analysis revealed that only the PCR product of HVVMRXALLeA0313H07_3 had SNP which can be exploited for CAPS marker development. By using dCAPS finder program, enzyme MboII was suggested to cut at the SNP position. However, no polymorphism was observed after digestion using MboII (Figure 3.1).

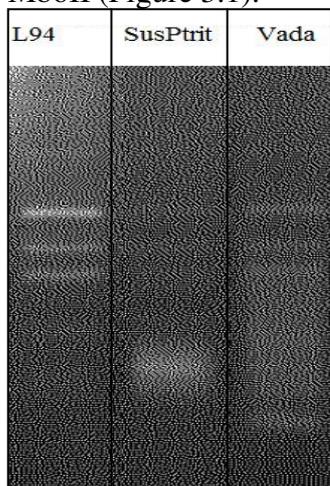


Figure 3.1 Profile of marker (HVVMRXALLeA0313H07_3) developed for *Rnhq*

3.3 DISCUSSION

The SCAR dominant marker did not map into the region of interest. This could be due to scoring error. For a SCAR dominant marker, the polymorphism is observed as presence and absence of PCR product. The absence of PCR product may be due to PCR failure or it is due to the polymorphism. Hence scoring error may occur.

During the screening of barley BAC library using the peak marker of *Rnhq*, 3 unique BAC contigs were picked. No further information is available to indicate which contig is positioned at the long arm of chromosome 7H where *Rnhq* is situated. It could be that two of the 3 BAC contigs were false positive when the BAC library was screened using the peak marker. The SCAR dominant marker obtained maybe is in one of the false positive contigs.

For HVVMRXALLeA0313H07_3, no polymorphism was observed even though the recommended enzyme was used for digestion. This may be due to that the differences between L94, SusPtrit and Vada were too little to show an obvious polymorphism resolved on gel electrophoresis.

For future study of generating molecular markers for *Rnhq*, BAC end sequences are still an important resource. It is worth to have another test by searching from different regions on the BAC clones.

3.4 CONCLUSION

One SCAR marker (HVVMRXALLeA0361C16b) was found. It shows polymorphism between L94/SusPtrit and L94/Vada. However, it was mapped into another region outside the target QTL. Thus, it cannot be used in fine mapping of *Rnhq*. SNP was found in HVVMRXALLeA0313H07_3, however, no polymorphism was observed after digestion, hence no CAPs marker was obtained. To sum up, fine mapping of *Rnhq* was not able to proceed.

CHAPTER 4 THE EFFECT OF PARTIAL RESISTANCE AND NON-HOST RESISTANCE QTLs TOWARDS HOMOLOGOUS AND HETEROLOGOUS RUSTS

4.0 INTRODUCTION

As explained in Chapter 1, partial resistance and non-host resistance are based on shared principles due to the evidence that genes for partial resistance seem to play similar roles as those governing non-host resistance in basal resistance. Moreover, based on histological studies partial resistance is prehaustorial similar to the mechanism of non-host resistance of barley to heterologous rust fungi

In order to reveal the association between partial resistance and non-host resistance, NILs in the background of SusPtrit, developed for partial resistance QTLs *Rphq2*, *Rphq3*, *Rphq11*, and a non-host resistance QTL, *Rnhq* were generated by crossing a donor parent with SusPtrit (Table 4.1). After several repeated backcrosses of progeny with the recurrent parent and selection, the NILs generated contained most of the SusPtrit genome except for a small chromosomal region containing an interesting gene or QTL. Development of such NILs for QTLs allows the evaluation of the target QTLs in a nearly uniform genetic background, overcoming the difficulties of identifying phenotypes of QTL (Marcel *et al.* 2007).

The purpose of this experiment is to evaluate macroscopic and microscopically the partial resistance and non-host resistance of barley against homologous rust (*P. hodei*. 1.2.1) and two heterologous rusts (*P. hodei-secalini* and *P. hodei-murini*). The quantification can provide a preliminary indication of association between partial resistance and non-host resistance.

4.1 MATERIAL AND METHOD

4.1.1 PLANT MATERIALS

QTL-NILs with the SusPtrit background (Table 4.1) were used in this experiment. Parental line for each NIL, SusPtrit, L94 and its NILs (L94-*Rphq2* and -*Rphq3*), Vada and its NILs (Vada-*rphq2*, and -*rphq3*) were included as reference. With each QTL-NIL, around 8-10 seeds were sowed, and 6 seedlings were remained to represent each QTL during evaluation, except the reference lines where only 4-5 seeds were sowed and two of them were kept for the experiment. One seedling of each QTL was used for microscopic assay, and other five seedlings of NILs and one seedling of the reference lines were used for macroscopic evaluation.

Table 4.1 plant materials used in this study and the QTLs presented in each line

Lines	QTL	Lines	QTL
Parental lines		L94 NILs	
Vada	<i>Rphq2</i> and <i>Rphq3</i>	L94- <i>Rphq2</i>	<i>Rphq2</i> and <i>rphq3</i>
L94	<i>rphq2</i> and <i>rphq3</i>	L94- <i>Rphq3</i>	<i>rphq2</i> and <i>Rphq3</i>
Dom	<i>Rphq16</i>	SusPtrit NILs	
Steptoe	<i>Rphq11</i>	<i>Su-Rphq2-BC5S2</i>	<i>Rphq2</i>
Morex	(Reference)	<i>Su-Rphq3-BC6S2</i>	<i>Rphq3</i>
Rec	(Reference)	<i>Su-Rphq11-s.F2.BC5S2</i>	<i>Rphq11</i>
SusPtrit	(Reference)	<i>Su-Rphq16-BC6S2</i>	<i>Rphq16</i>
Vada NILs		Host plant of heterologous rust	
Vada- <i>rphq2</i>	<i>rphq2</i> and <i>Rphq3</i>	<i>H.murinum</i>	(Reference)
Vada- <i>rphq3</i>	<i>Rphq2</i> and <i>rphq3</i>	<i>H.secalini</i>	(Reference)

4.1.2 INOCULUMS AND DISEASE TEST

Three species of rust fungi were used during infection process (Table 4.2). The inoculation was carried out with fresh collected spores supplemented with spores from the liquid nitrogen storage to obtain a sufficient amount of 8 to 9 mg for the heterologous rusts and 3 mg for the homozygous rust to be used for inoculation. After the inoculation (as described in Chapter 2), LP50S was scored for the disease test, and RLP50S was set as parameter to evaluate the effect of partial resistance towards homologous rust. For heterologous rusts, Infection Frequency (IF) and Visible Infection frequency (VIS) were scored on the 13 day after inoculation. IF and VIS of each line relative to SusPtrit (RIF and RVIS) were used as parameters with non-host resistance towards heterologous rusts. The IF and VIS formulas are described as:

IF= infection sites of heterologous pustules / S

S= the leaf area (cm²)

VIS= IF + flecks/S

Flecks = immature flecks on the leaf

Table 4.2 Rust isolates used in this study

Pathogens	Host plant	Heterologous/ Homologous Rust	Common name
<i>P. hordei isolate 1.2.1</i>	<i>Hordeum vulgare</i>	Homologous	Barley leaf rust
<i>P. hordei-murini</i>	<i>H. murinum</i>	Heterologous	Wall barley leaf rust
<i>P. hordei-secalini</i>	<i>H.secalinum</i>	Heterologous	Meadow barley leaf rust

4.1.3 SLIDE PREPARATION AND HISTOLOGICAL EVALUATION

Five days (*P.hordei 1.2.1*) and 7 days (*P.hordei-murini*, *P.hordei-secalini*) after inoculation, about 2-3 cm middle part of the leaf segment for each line were collected and put in acetic acid/ ethanol (1:3). The fluorescence staining was performed according to Rohringer *et al.* (1977), except that Calcofluor was replaced by Uvitex 2B (CibaGeigy) (Appendix 2). Leaf segments after staining were put on a microscope slide in 100% Glycerol in such a way that the longitudinal axis of the leaf and hence the rows of stomata are parallel to the longer axis of the slide.

Observation was done from the main vein or from one of the corners when the leaf was screened. Around 50 infection units were evaluated on each segment and classified according to their stage of development (Table 4.3) (Niks and Kuiper, 1982a; Niks, 1982b). Infection process of leaf rust to barley can be illustrated in figure 4.4(Kuijken, 2009). Cells at infection sites were classified as necrosis if they showed yellow or browning of the cell contents. The size of the colonies was assessed with an eyepiece micrometer by measuring the longest diameter. The outmost stomatal rows were excluded from observation to avoid possible effects. If the pathogen failed to develop in the stomata, it can also be ignored for scoring. The data collected were analyzed by using SPSS and Excel.

Table 4.3 Designation and definition of the infection units of leaf rust in barley, according to their stage of development (Niks and Kuiper, 1982a)

Development phase	Abbreviation	Designation of the infection unit	Definition
Nonpenetration	NP	Nonpenetrant	Appressorium over stoma (not cell wall) without formation of a substomatal vesicle
Substomatal vesicle formation	SSV	Aborted SSV	SSV without hyphae
Early Abortion with/without necrosis	EA	Early aborted colony	SSV with primary infection hyphae and up to six haustorial mother cells
Establishment	Establishment	Established or late-aborted colony	Branched hyphae, six or more haustorial mother cells

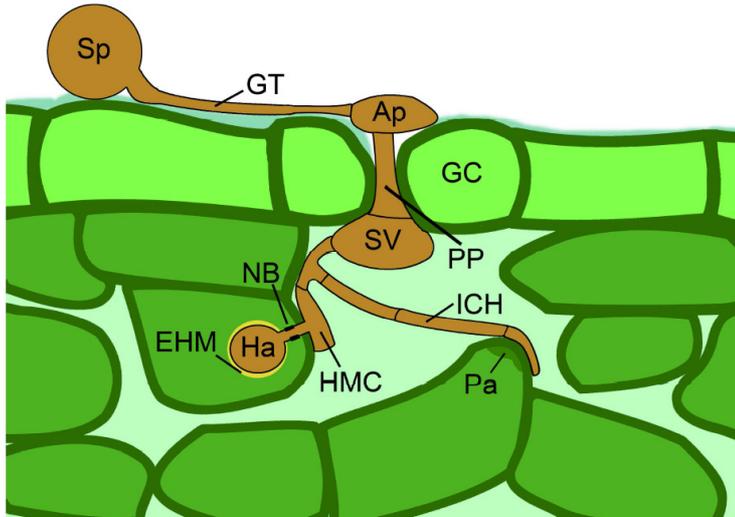


Figure 4.4 Invasion of leaf tissue by a rust spore in successive order (Kuijken, 2009)

Hydration and germination of the spore (SP) search for a stomatal opening by the germ tube (GT). Formation of an appressorium (Ap) and forcing through the guard cells (GC) by the penetration peg (PP). Formation of the substomatal vesicle (SV) from which intercellular mycelium (ICH) arises. After sensing the wall of the plant cell, a haustorial mother cell (HMC) is formed. A neckband bridges the plasma membrane of fungus and plant cell. The extrahaustorial matrix (EHM) is a newly formed membrane by the host plant cell and surrounds the feeding and putative communication organ, the haustorium (Ha). Intrusion of the cell by the pathogen can be prevented by the formation of cell wall reinforcements also known as papillae (Pa).

4.2 RESULT

4.2.1 EFFECT OF PARTIAL RESISTANCE AND NON-HOST RESISTANCE QTLs TOWARDS HOMOLOGOUS RUST

The performance of the reference lines were as expected (Marcel, 2007; Yeo, 2008) with two replications, supplemented with another two replications from an MSc student using the same method (Dido, 2010), except for that *L94-Rphq2* had relatively lower values compared with it was tested in the previous study (Thierry, 2006). This may indicate that the disease test experiment was confirmed to be consistent in the analysis of target QTLs.

According to previous experience on the performance of reference lines, Vada should have the highest RLP50S while L94 and SusPtrit having the lowest RLP50S. Based on the RLP50S, all the QTL-NILs had significantly (P value < 0.05) higher RLP50S compared to SusPtrit (Figure 4.5). *Rphq2* had an effect on partial resistance of *Su-Rphq2-BC5S2*, which increases the latency period by 3.5 % compared to SusPtrit. The effect is also observed in *L94-Rphq2*. *Rphq2* in *Vada-rphq3* seems to perform as the expected result.

SusPtrit NIL of *Rphq3* performed 2% higher in RLP50S compared to SusPtrit. Thus, *Rphq3* also contributed to the effect on partial resistance of *Su-Rphq3-BC6S2*. Similar effect of resistance was also observed in *L94-Rphq3* and *Vada-rphq2*.

Rphq11 and *Rphq16* as introduced in Chapter 2 are partial resistant QTLs effective at the seedling stage. Data in this study showed that they enhanced partial resistance of *Su-QTL11.s-F2.BC5S2* and *Su-Q16-BC6S2* up to 3% and 4%, respectively.

Rnhq, a non-host resistance QTL also showed an effect at *Su-Qnh.v-F2.BC5S2* towards homologous rust. The RLP50S was raised 1% compared to SusPtrit.

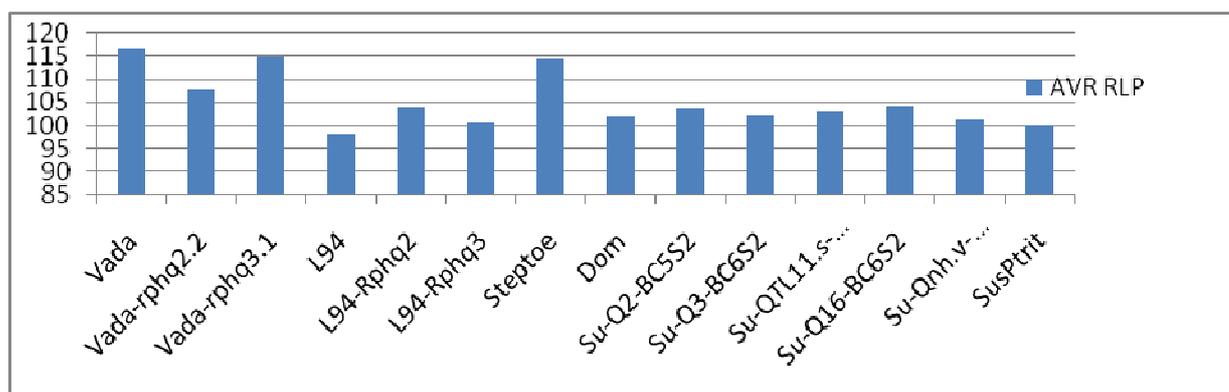


Figure 4.5 RLP50S of QTL-NILs and reference lines towards *P. hodei 1.2.1* at seedling stage

4.2.2 EFFECT OF PARTIAL RESISTANCE AND NON-HOST RESISTANCE QTLs TOWARDS HETEROLOGOUS RUST

The experiment was performed with only one replication, and the performance was not ideal due to experimental error and time limitation. To analyze the effect of target QTLs towards heterologous rust, another 2 replications using the same methodology from previous student (Dido, 2010) were used. Six parameters (RIF, RVIS, NP, EA, Establishment and the colony size) (Table 4.3) were scored in each replication as shown in Figure 4.7.

Concerning phenotypic data, RIF and RVIS (Figure 4.7 a, b), NILs of targeted QTLs were having significantly lower values compared with SusPtrit. Among those, the lowest values for RIF and RVIS were observed in lines with *Rphq11* and *Rphq16*, respectively.

The microscopic observation revealed that the proportion of NP of each SusPtrit NIL was significantly (P value < 0.05) higher than SusPtrit's for Phm (Figure 4.7 c). The resistance of SusPtrit NILs towards Phs did not display significantly higher values. The highest average value of NP was observed in *Su-Rphq2-BC5S2* (15%) for both Phs and Phm.

Based on EA, the SusPtrit NILs had significantly (P value < 0.05) higher EA proportion compared to SusPtrit (Figure 4.6 d), except that *Su-Qnh.v-F2.BC5S2* when challenged with Phm. The highest EA was found in *Su-Q2-BC5S2*.

In corresponding to the proportion of EA, all the SusPtrit NILs had significantly (P value < 0.05) lower proportion of established colonies for Phm and Phs compared to SusPtrit (Figure 4.6 e). *Su-Rphq2-BC5S2* had the lowest proportion of established colonies for Phs and Phm.

Established colonies in each QTL-NIL line had significantly (P value < 0.05) smaller sizes comparing to SusPtrit in Phm (Figure 4.6 f). Whereas in Phs, no significant difference was observed.

Rphq2 had an effect on non-host resistance of *Su-Q2-BC5S2*, which decreased the RIF up to 56% and 69% towards Phm and Phs, respectively. And RVIS of *Su-Q2-BC5S2* towards Phm and Phs was also reduced by 49% and 33%, respectively. SusPtrit NIL of *Rphq2* in microscopic assay also displayed non-host resistance towards heterologous rust. NP of *Su-Q2-BC5S2* was brought up 3% and 8% for Phs and Phm, respectively. For EA towards Phs and Phm, *Rphq2* raised the proportion up to 37% and 22%, respectively. In correspondence with EA for Phs and Phm, Established colonies' percentage declined by 38% and 30%, respectively. 4 μ m smaller colony sizes were observed both for Phs and Phm compared with SusPtrit's. The effect was also observed in *L94-Rphq2* and *Vada-rphq3*, which seem to exhibit expected result. As *Vada* is immune to heterologous rust, *Vada-rphq3* presented much obvious difference with SusPtrit NIL.

An effect on non-host resistance of *Su-Q3-BC6S2* from *Rphq3* also lessened the RIF by 56% and 60% for Phs and Phm, respectively. In RVIS, the decrements were 31% and 44% for Phs and Phm, respectively. In microscopic assay, 1% and 5% NP percentage reduction were observed for Phs and Phm, respectively. For EA towards Phs and Phm, *Rphq3* expanded the proportion up to 32% and 28%, respectively. Corresponding to EA for Phs and Phm, established colonies' percentage diminished

by 38% and 29%, respectively. 5µm and 4µm smaller size of colonies was detected for Phs and Phm, respectively. *L94-Rphq3* and *Vada-rphq2* appeared to achieve expected result.

Rphq11 enhanced non-host resistance of *Su-QTL11.s-F2.BC5S2* towards Phs and Phm for 69% and 75% abatement of RIF, respectively. And 57% and 70% were abated for Phs and Phm in RVIS, respectively. For microscopic detection, 3% and 6% NP was increased for Phs and Phm, respectively. 31% and 19% more EA was detected in *Su-QTL11.s-F2.BC5S2* for Phs and Phm, respectively. In correspondence with EA, 34% and 25% established colonies were reduced for Phs and Phm, respectively. 5µm and 4µm smaller size of colonies was detected for Phs and Phm, respectively.

Non-host resistance of *Su-Q16-BC6S2* towards Phs and Phm were revealed by 61% and 58% reduction of RIF, respectively, compared with SusPtrit. 52% and 43% less RVIS were also observed for Phs and Phm, respectively. *Rphq16* also contributed to non-host resistance, revealed by microscopic illustration. 3% and 7% NP were enhanced for Phs and Phm, respectively. For EA, 29% and 11% more were measured for Phs and Phm, respectively. In turn, established colonies were reduced by 32% and 19% for Phs and Phm, respectively. Colony sizes were 3µm and 4µm smaller compared with SusPtri for Phs and Phm, respectively.

Non-host resistance QTL, *Rnhq*, contributed to the non-host resistance of *Su-Qnh.v-F2.BC5S2*, showing that 59% and 61% reduction compared with SusPtrit of RIF for Phs and Phm, respectively. And also evidence was found that 42% and 49% of RVIS were cut back for Phs and Phm, respectively. Microscopic assay revealed that, 1% and 5% of NP were increased, as well as 17% and 4% of EA were increased for Phs and Phm, respectively. In correspondence with EA, established colony proportion was declined by 7% and 10% in contrast with SusPtrit, for Phs and Phm, respectively. Colony sizes were 3µm and 4µm smaller compared with SusPtri for Phs and Phm, respectively.

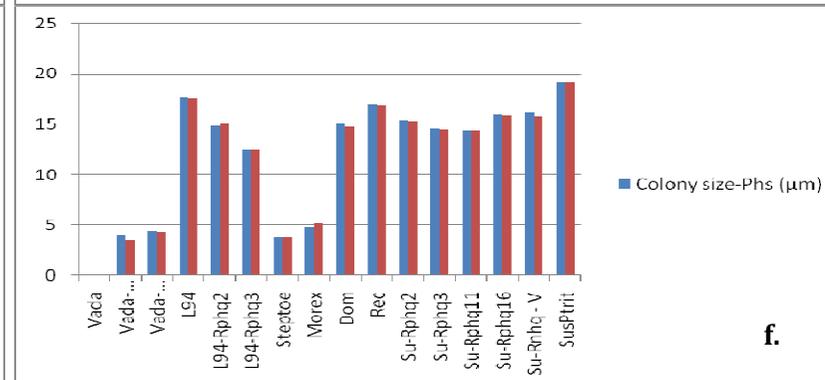
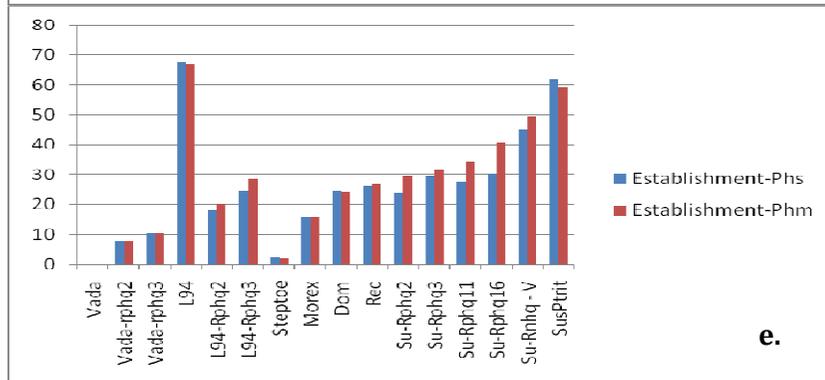
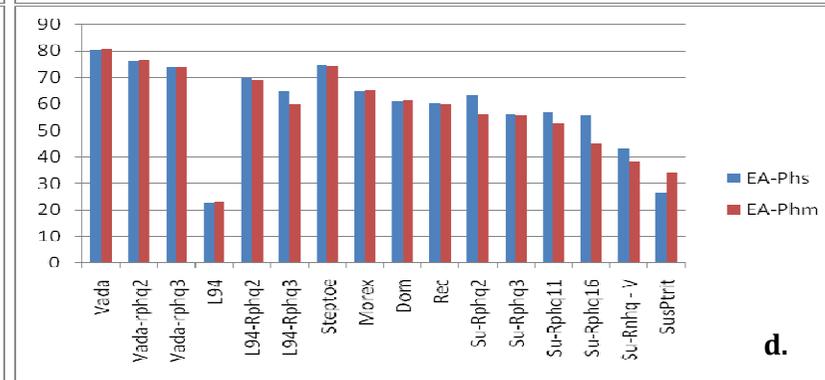
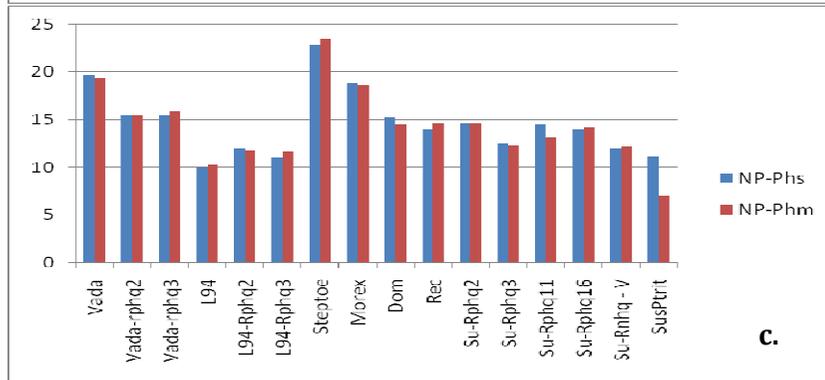
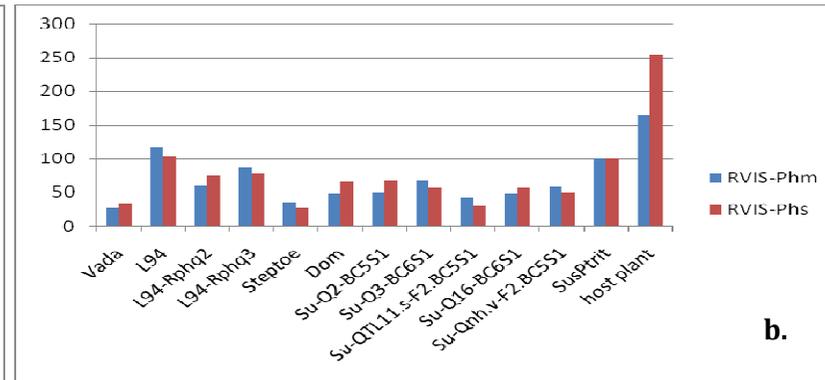
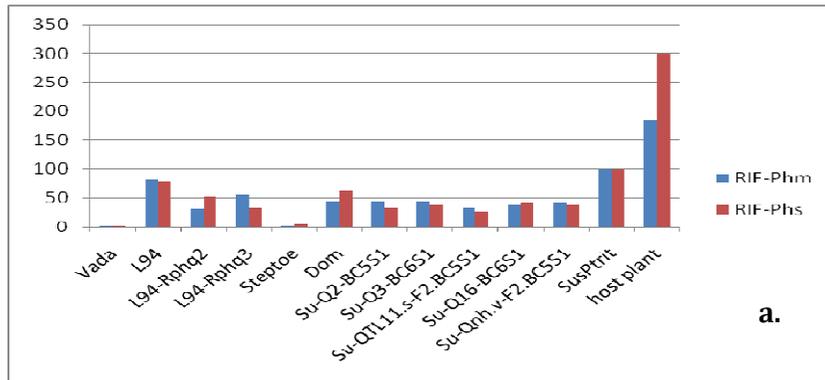


Figure 4.7 Data of effect to heterologous rust. **a** and **b**. RIF and RVIS of Phm and Phs on QTL-NILs and reference lines at seeding stage. **c**, **d** and **e**. proportion of each develop stage of rust (NP EA and Establishment) of Phm and Phs on QTL-NILs and reference lines at seeding stage. **f**. colony size of infection units of Phm and Phs on QTL-NILs and reference lines at seeding stage.

4.3 DISCUSSION

This chapter reveals that partial resistance QTLs, *Rphq2*, *Rphq3*, *Rphq11* and *Rphq16* and the non-host resistance QTL, *Rnhq*, in barley have effects of partial resistance towards homologous leaf rust.

All target QTLs also have effects towards heterologous rusts, as illustrated from some parameters showing significant difference between QTL NILs and SusPtrit. RIF and RVIS from phenotypic prospective are both good parameters in this study. *Rphq11* and *Rphq16* had the largest effect towards two heterologous rusts (Phs and Phm) compared with others shown in data of RIF and RVIS, respectively.

Microscopic assay is a good tool in combination with genetic experiments, for better revealing rust development stages and research on quantitative resistance. Partial resistance of barley to *P. hordei* was proven to occur between substomatal vesicle formation and haustorium formation (Niks, 1981; Niks, 1987). The same holds true for the mechanism of non-host resistance of barley to heterologous rust species (Dijk, 2007).

Among the parameters used for microscopic observation NP and colony size is not a good parameter to evaluate the resistance towards Phs. No significant difference between QTL-NILs and SusPtrit was observed.

Proportion of EA and Established colonies are good parameters and significantly correlated (P value= 0.000). Early abortion of colonies indicates that the fungus had not been able to develop a successful haustorium, and hence, reflects pre haustorium (Niks, 1983). Thus, high EA leads to low percentage of Established infection sites.

To discover the possible association between partial resistance and non-host resistance, the correlation of the effects between partial resistance QTLs and non-host resistance QTL towards homologous and heterologous rusts was analyzed. RLP50S and EA are two good parameters chosen from each type of resistance test. There are significant (P value< 0.01) and positive correlations between RLP50S, EA towards Phs and Phm (Figure 4.8).

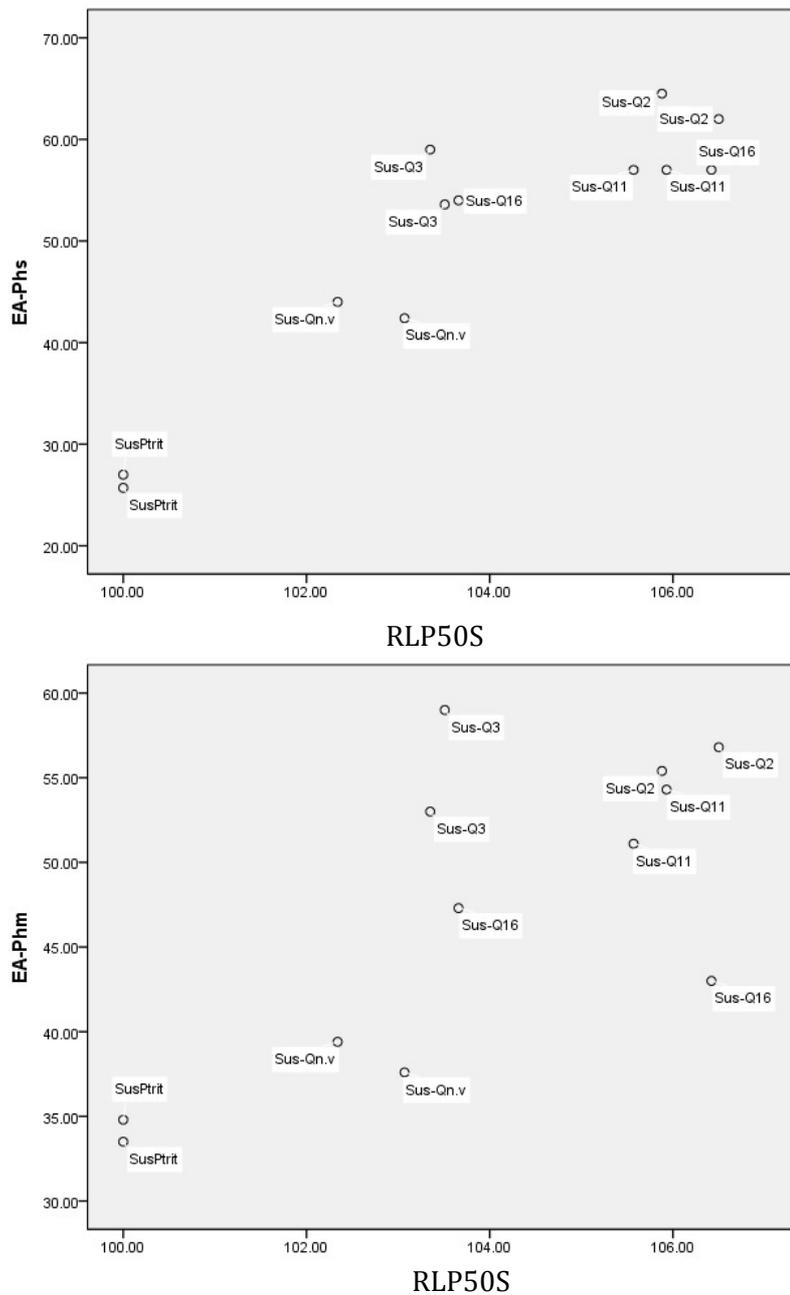


Figure 4.8 correlation between percentage of early aborted (EA) infection units in each QTL-NIL and SusPtrit towards Phs and Phm, and relative latent period (RLP50S) of homologous rust.

Based on this result, there is an association between partial resistance and non-host resistance. However, this study is at QTL level. At gene level, it could be the same gene or different genes are involved. Hence, the association may not be true.

4.4 CONCLUSION

In this study, it was found that partial resistance QTLs, *Rphq2*, *Rphq3*, *Rphq11* and *Rphq16* and the non-host resistance QTL, *Rnhq*, have effects on partial resistance towards homologous leaf rust *P. hordei* isolate 1.2.1 and heterologous rusts, *P. hordei-murini* and *P. hordei-secalini*.

A significant positive association was found between parameter of RLP50S and EA, which indicated that there is a possible association between partial resistance and non-host resistance.

Study on effect of basal resistance can help to accumulate the effective QTLs into cultivars of interest to create an artificial (near) non-host cultivars, providing a high level of durable resistance against specific pathogen.

CHAPTER 5 GENERAL CONCLUSION

This thesis researched on three different experiments which together contribute important information to one major research project on the association between host and non-host basal resistance. Genes found for basal resistance are quantitative, which can provide durable resistance compared with qualitative resistance genes. The knowledge exploited on the genetic characterization and function of these genes will help breeding for resistance of barley and other crops.

Chapter 2 intended to identify genes involved in basal resistance, which will enable molecular markers development in the future study, and verify responsible genes quickly. The “fast and dirty” approach in this experiment by using homozygous recombinant lines is applicable to fine *Rphq11* and *Rphq16* which speeds the fine mapping process. *Rphq11* was fine mapped into two genetic windows. One is between WBE144 and K14 (0.1 cM), and its candidate genes are glutathione peroxidase and serine racemase. The other genetic window flanked by GBMS244 and WBE130 (0.4 cM), with the candidate genes of protein kinase and SHR5-receptor-like kinase. Based on the synteny with rice, the physical sizes of its two genetic windows are about 22kb and 43kb.

The genetic window of *Rphq16* was fine mapped into the region between ABC11948_3 and TC181991_2 (3.1 cM) which is 7.4 cM narrower than it is in previous study. The candidate genes found in the genetic window are oxidoreductase and glutathione S-transferase. The physical size of the genetic window of *Rphq16* is approximately 175kb. In order to further fine map and study these target QTLs, development of more molecular markers in obtained regions is needed.

In Chapter 3, development more molecular markers is needed for fine mapping of *Rnhq*, a non-host resistance (non-host basal resistance) QTL in barley. One SCAR marker (HVVMRXALLeA0361C16b) was found. It shows polymorphism between the parental lines L94/SusPrit and L94/Vada. However, after processing mapping of the marker, it was located into another region outside the target QTL. Thus, it cannot be used in fine mapping of *Rnhq*. Additionally, SNP was found in HVVMRXALLeA0313H07_3, however, no polymorphism was observed after digestion, and hence no CAPS marker was obtained. In addition, fine mapping *Rnhq* can not be executed in this study.

In chapter 4, near isogenic lines (NILs) of targeted partial resistance and non-host resistance QTLs were used to test the specificity of these QTLs towards homologous and heterozygous leaf rusts. In this study, it was found that partial resistance QTLs, *Rphq2*, *Rphq3*, *Rphq11* and *Rphq16* and the non-host resistance QTL, *Rnhq*, in barley, have obvious effects towards homologous leaf rust *P. hordei* isolate 1.2.1 as well as heterologous rusts, *P. hordei-murini* and *P. hordei-secalini*.

A significantly positive association was found between parameter of RLP50S and EA, which indicated that there is a possible association between partial resistance and non-host resistance.

Study on the effect of basal resistance can help to accumulate the effective QTLs into host cultivars, which could be turned into artificial (near) non-host, providing high level of durable resistance against specific pathogen.

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APPENDIX

Appendix 1 PCR conditions used for the development of SCAR and CAPS markers and digestion profile

a) PCR mix used for the development of SCAR and CAPS markers

Components	Volume (μL) 1 x
H ₂ O (autoclaved)	18.45
PCR buffer Dream taq (10x)	2.5
dNTPs (5mM)	1.0
Forward primer (5 pmol/ μ L)	1.0
Reverse primer (5 pmol/ μ L)	1.0
Dream taq polymerase (5 units/ μ L)	0.05
DNA	1.0
Total volume	25

b) Digestion profile

Components	Volume (μL) 1 x
H ₂ O (autoclaved)	
PCR product	3
Enzyme	
Total volume	15

Appendix 2 protocol of staining for microscopic evaluation

1. Put newly collected leaf samples in tubes of acetic acid/ ethanol (1:3) for overnight. Label the tubes with paper written by pencil, which cannot be solved by alcohol or by water.
2. Wash 1 x 30 min in ethanol (50%)
3. Wash 1 x 30 min in 0.05 N NaOH (2g/l)
4. Rinse 3 x water
5. Soak for 30 min in 0.1 M Tris/HCL buffer (pH 8.5) ¹⁾
6. Stain for 5 min in a solution of 0.1% Uvitex in the same buffer, or for 10 min in a solution of 0.05% Uvitex in the same buffer.
7. Rinse thoroughly 4 x in water
8. Wash for 30 min in a solution of 25% glycerol.

¹⁾ Preparation of 0.1 M Tris/HCL buffer (pH 8.5):

Dissolve 12.1 g Tris in 88 ml H₂O

Adjust pH to 8.5 with HCL (25%)