# ASSOCIATION MAPPING AND GENEALOGY STUDY OF GENES FOR RESISTANCE TO PATHOGENS IN BARLEY

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*"If we knew what it was we were doing, it would not be called research, would it?"*

(Albert Einstein)

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# SUMMARY

Association Mapping is an alternative approach to the conventional genetic analyses that aims on describing the genetic basis of simple and complex traits. Developed in the first place for human genetics, this method was recently applied to plants.

The objective of this study was to continue the work of Arnold Kraakman (2005) at the Department of Plant Breeding of The Wageningen University on the association mapping of agronomical, morphological and resistance traits in a collection of modern spring barley cultivars. The idea was to create a new integrated map composed of the markers used by Kraakman et al. mapped corresponding to their position in a barley consensus map recently published, collect data relative to complete and partial resistance against barley powdery mildew (*Blumeria* graminis f.sp. *hordei*) and search for the correlation between the markers of this new map and the traits from the work of Kraakman et al. and the powdery mildew testing. As a result, a new integrated map of 131 markers was produced. The results of the association with Kraakman's traits were in agreement with his findings. Additionally, reported and non reported Quantitative Trait Loci (QTLs) and major genes for powdery mildew resistance and for the other traits were positioned in the new integrated map.

In the second part of this thesis, we tried to find the source of a new gene for tolerance to the Barley Yellow Dwarf Virus (BYDV), also reported by Kraakman at al. in 2006. By a literature review of the genealogy of the fourteen cultivars of the collection reported to carry this gene, ancestor candidates were selected. A molecular testing, using a SSR marker reported to be highly associated to this gene, allowed confirming that the British cultivar 'Midas', released in 1970, was the source of this gene for seven out of the fourteen cultivars.

This study is an illustration of some possibilities offered by the association mapping approach for the analysis of the genetic basis of qualitative and quantitative traits in barley.

# 1. GENERAL INTRODUCTION

## 1.1. Breeding for partial resistance in barley

In barley, as for all the other major cultivated crops, complete monogenic resistances are frequently overcome by pathogens, sometimes ruining the result of a decade of breeding efforts. This is especially the case for obligate biotrophs like *Puccinia hordei* (barley leaf rust) of *Blumeria graminis* f. sp. *hordei* (barley powdery mildew) and viruses that can breakdown monogenic resistances in only few years after release of the variety. One reason of this phenomenon is that complete monogenic resistances induce a high selection pressure on the pathogen population in favor of the individuals that overcome the resistance by mutation of the corresponding avirulence factor. As a consequence, researches are more and more focusing on a more durable type of resistance: Partial Resistance (PR). Partial resistance mechanisms are not inducing a high selection pressure on the pathogen as they do not prevent infection but reduce its severity. Moreover, its polygenic character makes it much more difficult to overcome for the pathogen. In those conditions, breeding companies and institutes are more and more interested by this type of resistances and more and more researches are dedicated to the analysis of the genetic basis of partial resistance using molecular methods.

## 1.2. Mapping of Quantitative Trait Loci (QTLs) for partial resistance in barley

In barley genome mapping, thousands of molecular markers have been developed and mapped (Marcel et al., 2007) and several extensive linkage maps have been produced (Karakousis et al., 2003; Liu et al., 1996; Marcel et al., 2007; Qi et al., 1998; Ramsay et al., 2000) allowing to describe major genes and QTLs for many traits in different genetic backgrounds (Shtaya et al., 2006). Mainly, the efforts have been focused on traits such as grain yield, malting quality and disease resistance and have lead to major improvement in the comprehension of the genetic basis of those quantitative trait (Mather, 1999).

Recently, an alternative approach of genetic analysis, developed for human genetic, was adapted to gene and QTL mapping in plants. This technique, called Association Mapping, presents many advantages over the conventional techniques and is, therefore, predicted to become ‘a powerful tool for analysis of disease resistance genotype x pathotypes x environment interactions’ (Williams, 2003).

The following study is based on one of those association studies performed between 2001 and 2005 by a team of researchers from the Department of Plant Breeding of Wageningen University leaded by Dr. Arnold Kraakman (PhD thesis, Wageningen university, 2005).

# 2. ASSOCIATION MAPPING OF POWDERY MILDEW RESISTANCE IN A COLLECTION OF TWO-ROW SPRING BARLEY CULTIVARS

## 2.1. INTRODUCTION

### 2.1.1. Definitions and precedents

Linkage Disequilibrium (LD) correspond to the genetic phenomenon of non-random association of alleles at different sites of a genome (Remington et al., 2001). Association mapping is an approach of genetic mapping making use of LD to study association between markers and traits, represented by the covariance of a marker polymorphism and a trait of interest (Gupta et al., 2005) in a population of genetically distant individuals. This population can be, for example, a collection of varieties which are separated by several generations of breeding.

The level of LD between locus (markers, genes or QTLs) can be influenced by many factors, increasing (inbreeding, small population size, natural selection) or decreasing (out-crossing, high mutation rate) the linkage between loci. One of those factors is the population structure. A population is said structured when the frequency of finding a trait in a sub-population is significantly different from finding it in another sub-population. For example, if the population is structured on a geographical basis, there is more chance to find a trait like drought tolerance in cultivars grown in dry areas than in cultivars grown under other climatic conditions(Gupta et al., 2005). The level of LD between locus is called “Linkage Disequilibrium Distance (or Decay) (LDD)” It is a measure of how rapidly does the disequilibrium decays when the genetic distance between two loci increases. It varies significantly between species, but also within species, between subspecies or populations. It is an important measure for association studies as it indicates how dense the genetic map must be, in term of number of markers per Centimorgan (cM), in order to efficiently describe the marker-traits associations in a population. Therefore, the LDD is always evaluated at an early stage of an association study by measuring the significance of correlation between the markers of the map.

### 2.1.2. Association study from Kraakman et al. (2006)

In 2006, Kraakman studied the opportunities offered by the method of association mapping (also called linkage disequilibrium mapping) in order to describe the genetic basis of agronomical, morphological and resistance trait in barley during his PhD thesis at the Department of Plant Breeding of Wageningen University in 2001-2005. For this purpose, they created a collection of 148 modern barley cultivars by collecting varieties that were used all over Western Europe for the last 15 years and they collected data about traits in those varieties from multi-environment trials (Kraakman, 2005; Kraakman et al., 2006; Kraakman et al., 2004). The traits included in this study were Days To Heading (DTH), Plant Height (PH), leaf rust resistance (infection type (IT), Area Under the Disease Progress Curve (AUDPC) and latency period (LP)), tolerance to barley yellow dwarf virus (BYDV), rachilla hair length and lodicule size (Kraakman et al., 2006).

Additionally, they genotyped a total of 286 AFLP on this collection. 50 of those markers were discarded as they didn't show enough polymorphism (less than 5 % frequency for the least represented allele) In the remaining 236 markers, 123 were mapped using three segregating populations and an integrated map was constructed (called Kraakman's map in this study). The other markers were unmapped. They evaluated the LDD to 10 cM in this collection (Kraakman et al., 2004) and didn't find any evidence of a population structure. Subsequently, 11 SSRs and 5 AFLPs were added in region where this integrated map was showing gaps. Gaps are defined as regions of the genome were markers are separated by more than twice the LDD and where, consequently, QTLs or genes cannot be mapped as they are too far from a marker to show linkage disequilibrium.

Finally, a statistical analysis of marker-trait association was performed in order to find marker-traits correlations reflecting the presence of a gene or QTL. An integrated map was published giving the position of 139 markers (128 AFLPs and 11 SSRs), many of them associated with one or several traits (Kraakman et al., 2006).

### 2.1.3. The barley consensus map

A consensus map of barley was constructed in 2006 by Marcel et al. in the Laboratory of Plant Breeding of Wageningen University. It is composed RFLP, AFLP and SSR markers mapped in three Doubled Haploid (DH) populations and three Recombinant Inbred Lines (RILs) populations. In total, the consensus linkage map contains 3258 markers, covering

1,081 cM. With an average distance of 0.33 cM between adjacent markers, this map present the highest density of marker reported so far in barley. It is a very valuable tool genetic studies in barley (Marcel et al., 2007) as it contains most of the reported markers available for genetic analysis in Barley.

### 2.1.4. Powdery mildew resistance in barley

Several laboratories have focused their researches on powdery mildew resistances during the last 15 years in several laboratories leading to the discovery of many resistance gene.(*Blumeria graminis* f.sp. h*ordei*) (Chelkowski et al., 2003; Saghai Maroof et al., 1994) Some of them, such as the *Mla* cluster or *mlo*, have been widely studied (Jorgensen, 1992), cloned and used in breeding programs during the last decade (Hovmoller et al., 2000; Jensen et al., 1992). However, due to the great variability of this pathogen, the monogenic resistances are becoming inadequate and research on quantitative resistance is needed to allow the breeders developing Marker Assisted Selection (MAS) strategies and produce new varieties showing durable resistance to this major pathogen of barley.

### 2.1.5. Research objective and questions

The main objective of this study was to create an integrated map containing markers mapped in the barley consensus map and used for the association mapping of traits in barley. This consensus map representing a very large source of markers, an association study based on correlations between traits and markers from this consensus map can be easily improved by adding markers selected from this consensus map and perform correlation test with additional traits.

In this study, the barley association mapping study of Kraakman was extended by adding data about powdery mildew resistance and performing a marker-trait association test.

Several research questions will have to be answered in order to evaluate if this objective can be reached. Those questions are: Is it possible to create a map with the markers used by Kraakman and that are reported into the barley consensus map, and this, without loosing too much information? Can partial resistance against barley powdery mildew be accurately studied by association mapping on this integrated map? Does the final result bring significant improvements of the previous association study?

## 2.2. MATERIAL AND METHODS

### 2.2.1. Plant material

The collection was composed of 148 modern two-row spring barley cultivars. They were homozygous diploids lines created by inbreeding or by doubling haploids. Those cultivars have been created by several breeding companies and grown all over Europe during the last 15 years. They represent a large part of the European germplasm used for the past 15 years (Kraakman et al., 2006; Kraakman et al., 2004).

This collection has been stored in a cold room at the Wageningen University (Wageningen, The Netherlands). The first task was to check the collection. To complete the collection, seeds of the missing cultivars were ordered to gene banks and breeding companies all over Europe and in USA. However, some cultivars could not be found back as no institution was found that could provide the seeds.

### 2.2.2. Powdery mildew resistance genes review of the collection

A review of the major gene for resistance to powdery mildew carried by each of the 148 cultivars was performed by combining information from databases (see References), webpages (see references) and literature (Hovmoller et al., 2000; Jensen et al., 1992; Ramsay et al., 2006).

### 2.2.3. Barley powdery mildew isolates

Two isolates of powdery mildew were used. The first one, called ‘common isolate’ (I1) is an isolate maintained for several years in the Department of Plant Breeding of Wageningen University. The virulence spectrum had not been studied but this isolate was known to be virulent to several major resistant genes (R genes) including the *MlLa* R gene. The second one, called ‘Danish isolate’, or isolate B4 (I2), is an isolate used in the department for indirect tests for partial resistance to leaf rust (*Puccinia hordei*), as an important QTL for the resistance against leaf rust is located very close to the *MlLa* gene to which this isolate is avirulent. As for I1 the virulence spectrum of this isolates had not been reported.

## 2.2.4. Disease evaluation at the seedling stage

### 2.2.4.1. Disease evaluation of the barley collection: seedling testing

A seedling test of infection was performed to evaluate the barley powdery mildew resistance response of the collection. Seedlings were grown in 34x39 cm plant boxes. On each box the cultivars were sown on three rows with eight cultivars per row. For the first replication, six seeds per cultivar were sown. For the next replications, the number of seeds planted was adapted depending on the quality of germination recorded in the previous replicates. The number of seeds planted per cultivar varied from three to more than ten for the cultivars showing very bad germination in the previous replications. Around 20 % of the collection was showing bad germination (no germination in two or more replicates). Most likely, the fungicide used by one of the companies to coat the seed was negatively affecting the quality of the seeds.

Twelve days after sowing, one segment from first leaf of each cultivar was sampled and put on an agar medium containing 125 ppm of benzimidazole in a polystyrene Petri dish. The leaf segments were placed in two windows cut in the agar with their extremities inserted into the media to prevent the material from drying. L94 and Manchuria were used as a reference in some of the replicates as L94 is known to be fully resistant to powdery mildew and Manchuria fully susceptible. The Petri dishes were placed into a settling tower and inoculated with fresh powdery mildew inoculum produced on young seedlings of the susceptible cultivar ‘manchuria’. A hemocytometer was placed with the samples in the settling tower to measure the quantity of inoculum spread on the plates. After an incubation period of 4 days in a growth chamber at 20$^{o}$C with a fluorescent light supplied for 12h per day, the Infection Frequency (IF) was recorded by counting the number of colonies per 2 cm$^{2}$.

When all the replicates had been performed, all the cultivars that had shown signs of infection in at least one of the replicate were tested to determine the Infection Type (disease severity test). For each isolate, those cultivars were sown in two rows on plant boxes. 14 days after sowing, the plants were fixed in horizontal position with metal pins and inoculated in a settling tower. 10 days after inoculation, the infection type (IT) was scored on a scale from 0 to 4, the types 0 to 3 corresponded to different level of resistance (incompatible interaction, hypersensitive response) and type 4 corresponded to susceptibility (compatible interaction, no hypersensitive response) (see pictures in appendix 2).

#### 2.2.4.2. Virulence spectrum of the isolates

The virulence testing was carried out by including the Pallas differential series into the disease severity testing (see above). This differential series is composed of 19 Near-Isogenic Lines (NILs) with "Pallas" as a recurrent parent (Jensen et al 1992). Each of the line carries only one or two specific R genes for resistance against powdery mildew.

Table 2.1. Pallas differential varieties used for the virulence spectrum study of the two mildew isolates.

| Pallas lines | Resistance gene(s) | Infection types(s) when resistant[1] |
|---|---|---|
| Pallas | *Mla8* | 0 |
| P01 | *Mla1, Mla(Al2)* | 0 |
| P02 | *Mla3* | 0-1 |
| P03 | *Mla6, Mla14* | 0, 2-3 |
| P04B | *Mla7, Ml(No3)* | 0-1, 2-3 |
| P08B | *Mla9* | 0 |
| P10 | *Mla12, Ml(Em2)* | 0 |
| P11 | *Mla13, Ml(Ru3)* | 0, 1-2 |
| P16 | *Mlk* | 1 |
| P23 | *MlLa* | 2-3 |
| P09 | *Mla10, Ml(Du2)* | 1, 2-3 |
| P12 | *Mla22* | 0 |
| P13 | *Mla23* | 2-3 |
| P19 | *Mlp* | 2 |
| P20 | *Mlat* | 2 |
| P21 | *Mlg, Ml(CP)* | 0-1 |
| P22 | *Mla5* | 0 |
| P24 | *Mlh* | 1 |
| P17 | *Mlk1* | 1 |

[1] Infection type corresponding to a resistant status of the tested cultivar (Jensen et al., 1992)

### 2.2.5. Creation of the integrated map

#### 2.2.5.1. Selection of the common markers

The first step in the creation of the new integrated map consisted of comparing the data from the two maps: The lists of AFLP and SSR markers from Kraakman's map and from the barley consensus map were cross examined. The markers from Kraakman's map that were not positioned in the consensus map were discarded. The markers common to the two maps were selected if they were showing a sufficient level of polymorphism in the collection (more 5% of frequency for the least common allele). The markers were considered identical between the two maps when they had the same name (same E/M primer combination), when they were

mapped on the same chromosome and at comparable positions and when the difference between their migration distances (given by the last number in their name) was inferior to 5 base pairs  Finally, as the two integrated maps had been constructed using a L94 x Vada segregating population, only the markers amplifying for the same parent in the two maps were conserved. Markers that showed a contradictory pattern between the two maps were discarded. For example, a marker amplifying for Vada in Kraakman's map and for L94 in Marcel's individual map was discarded for showing contradictory patterns.

#### 2.2.5.2. Case of the unmapped markers

Almost half of the markers used by Kraakman were not positioned in the integrated map (113 out of 236) (Kraakman et al., 2004). But, many markers with the same or similar name to those unmapped markers could be found in the consensus map and were given a hypothetical position. However, there was a high probability of erroneous positioning especially for unmapped markers with slight differences in the migration distance (less than 5 bp). Therefore, only the most reliable markers, showing high correlation with their neighboring markers or with a trait located in the same region, were conserved. All the markers that remained doubtful were discarded.

#### 2.2.5.3. SSR markers filling the gaps

The common markers and the repositioned markers (unmapped in Kraakman's map) were gathered to create a so called 'backbone map'. But this map showed several gaps (regions where two adjacent markers were separated by more than 20 cM). Therefore, in order to produce a precise map covering all the genome, those gaps needed to be covered by adding new markers. Considering their interesting properties for QTL mapping, SSR markers were chosen to fill the gaps (Liu et al., 1996; Macaulay et al., 2001). As the backbone map contains markers mapped in the consensus map, the SSRs were also selected from the consensus map depending on their position, if they were mapped at least in L94 x Vada, their quality, their PIC value (level of polymorphism) and the availability of the primers (markers already available in the lab were preferred). In the end, a total of 27 SSR markers were selected.

## 2.2.6. Molecular work

### 2.2.6.1. DNA material

The DNA samples stored by Kraakman were used. When the DNA was missing or in insufficient quantity, it had to be extracted again. Plants of the corresponding cultivar were sown in individual pots with 4 or 5 seeds per pot (this number was increased when the germination was not successful). After 12 days, the first leaf was collected and the DNA extracted following the Retch protocol 1.2, based on the CTAB protocol (Saghai Maroof et al., 1984) scaled up to a multiple 96 racks with tubes. After isolation, random samples were taken from the extracted DNA samples and a general factor of dilution was chosen to level the concentration of all samples into a range of 50 to 200 ng after diluting with water. The DNA samples were stored in 96 wells PCR plates. Half of the DNA material was kept and stored. The other half was used for the molecular work. The DNA samples of the collection were distributed on two PCR plates, divided in 4 groups for the gel running (4 gels needed per marker).

### 2.2.6.2. SSR markers selected

In the 27 SSR primer combinations selected, 16 were directly available in the department of Plant Breeding of Wageningen University (code 1 to 97) and 11 were ordered and added to the list of SSR markers of the lab (code 114 to 133) (Table 2.3).

### 2.2.6.3. PCR mixture

Table 2.2. PCR mix composition per sample

| Components | Volume (µL) |
|---|---|
| MilliQ water | 6.60 |
| PCR buffer S-Taq (10x) | 1.00 |
| dNTPs (5 mM) | 0.40 |
| Primer Mix (F+R) (5pmol / µL) | 1.00 |
| Taq-polymerase (SuperTaq 5 U/ µL) | 0.05 |
| **Total Volume mix** | **9.00** |
| DNA | 1.00 |
| **Total Volume** | **10.00** |

After PCR, the reaction products were mixed with an equal volume (10 µl) of formamide loading buffer. The PCR plates were thermo-sealed and kept in the fridge at 4°C. Usually, the PCR reactions were done and the gel was run the next day. A better quality of results was observed when the plates were stored overnight instead of using it right after the PCR.

Table 2.3. Description of the 27 SSR added to the integrated map

| Code[1] | Name | Chr.[2] | PCR[3] | Position | Size range | PIC | F primer Unlabelled[4] | R primer IRD-labelled |
|---|---|---|---|---|---|---|---|---|
| 1 | Bmac0399 | 1H | C | 29 | 129-153 | 0.72 | CGATGCTTTACTATGAGAGGT | GGGTCTGAAGCCTGAAC |
| 5 | HvHVA1 | 1H | A | 102 | 134-136 | 0.34 | CATGGGAGGGGACAACAC | CGACCAAACACGACTAAAGGA |
| 8 | HVM36 | 2H | A | 31 | 110-128 | 0.77 | TCCAGCCGACAATTTCTTG | AGTACTCCGACACCACGTCC |
| 18 | Bmag0225 | 3H | B | 76 | 156-162 | 0.42 | AACACACCAAAAATATTACATCA | CGAGTAGTTCCCATGTGAC |
| 19 | Bmag0013 | 3H | B | 114 | 155-160 | 0.72 | AAGGGGAATCAAAATGGGAG | TCGAATAGGTCTCCGAAGAAA |
| 21 | HVM40 | 4H | A | 22 | 147-166 | 0.58 | CGATTCCCCTTTTCCCAC | ATTCTCCGCCGTCCACTC |
| 33 | Bmag0222 | 5H | B | 144 | 154-190 | 0.60 | ATGCTACTCTGGAGTGGAGTA | GACCTTCAACTTTGCCTTATA |
| 36 | Bmag0173 | 6H | B | 58 | 150 | 0.72 | CATTTTTGTTGGTGACGG | ATAATGGCGGGAGAGACA |
| 40 | Ebmac0806 | 6H | B | 75 | 168-178 | 0.78 | ACTAAGTCCTTTCACGAGGA | GTGTGTAGTAGGTGGGTACTTG |
| 50 | Bmag0841 | 3H | A | 91 | 108-125 | 0.62 | GGAAAGTACTTCAAACCTGAA | CTTACAAGATGATGAGAACGA |
| 66 | Ebmac0560 | 1H/5H/5H/6H | B | 59/44/48/62 | 222-225 | nd | CATATGTGTGCAAGTGTGC | AGATCCATGAGGTTGTGC |
| 81 | GBM1005 | 6H | A | 131 | nd | 0.28 | Confidential | Confidential |
| 83 | GBM1015 | 4H | A | 116 | nd | 0.78 | Confidential | Confidential |
| 89 | GBM1428 | 7H | A | 91 | 223-227 | 0.44 | CCCTCTACAGTCCGATCTGC | TAAACATGGCAACGAGGACA |
| 93 | scssr07106 | 5H | A | 20 | nd | 0.66 | GCGCTGTCTCTTCTATGTGC | AGGTGCTCCTAATCTGATGG |
| 97 | Bmag0500 | 6H | B | 32 | 157-187 | 0.80 | GGGAACTTGCTAATGAAGAG | AATGTAAGGGAGTGTCCATAG |
| 114 | GBM1482 | 4H | A | 37 | nd | 0.61 | GAGAGCTAGCCACCATTTGC | GACAGGTCGAGGCTGAGAAG |
| 117 | GBMS035 | 7H | C | 47 | 135-145 | 0.75 | Confidential | Confidential |
| 118 | GBMS062 | 1H | C | 21 | 124-128 | 0.68 | Confidential | Confidential |
| 121 | GBMS187 | 1H | C | 3 | 145-160 | 0.94 | Confidential | Confidential |
| 122 | GBMS190 | 4H | C | 80 | 125-131 | 0.79 | Confidential | Confidential |
| 126 | GBM1021 | 6H | A | 40 | nd | 0.72 | Confidential | Confidential |
| 129 | Bmag0357 | 5H | B | 65 | nd | nd | CTTCTACATCATCCTTGTTGC | ATGATCATTGTATTGAAGAGCA |
| 130 | Bmag0606 | 3H | A | 102 | nd | 0.87 | CTATTTGTAATGTATGTATGTCCC | TCATTGGTCCAGATAATACAA |
| 131 | EBmac0603 | 7H | B | 35 | nd | 0.74 | ACCGAAACTAAATGAACTACTTCG | TGCAAACTGTGCTATTAAGGG |
| 132 | HVM11 | 6H/7H | A | 88/135 | nd | nd | CCGGTCGGTGCAGAAGAG | AAATGAAAGCTAAATGGCGATAT |
| 133 | scssr10559 | 3H | A | 23 | nd | 0.65 | CATTTCCTCTCCCCTTGC | CTCACCTCCTGCCGATCC |

[1] code used in the lab, markers 1 to 97 were present in the lab, marker 114 to 133 were ordered during the study.
[2] Chromosome
[3] PCR profile used (see appendix 3)
[4] F: Forward, R: Reverse

#### 2.2.6.4. PCR conditions

Three different PCR profiles (appendix 3) were used depending on the primer combination (Table 2.3).

#### 2.2.6.5. Gel running

After being denatured at 95°C for 5 min, the DNA samples were loaded on a denaturing acrylamide gel. The gel was prepared by mixing 20 mL of P.A.A.(5% KB+), 15 µL of TEMED (N,N,N, Tetramethylethylenediamine) and 150 µL of 10% APS (Ammonium persulfate). Afterward, the mix was poured between two glass plates and left to polymerize for 45 minutes minimum. After cleaning of the glasses, TBE buffer diluted to 10% was used as an electrolyte solution. After warming and calibration of the DNA sequencer, the front of the gel was cleaned with a syringe filled the electrolyte solution to remove the urea "sweating" from the gel. Finally, a loading comb of 48 slots was inserted on top of the gel, 46 samples (one group, half of a PCR plate) of the markers labeled IRD 800, were loaded (0.4 to 1 µL of sample, using an 8 slot multi-channel pipette) and the corresponding labeled ladder was added on one of the remaining slot. After 5 minutes, the samples labeled 700 IRD were added with the corresponding ladder. As SSR were used, the gels were loaded up to 3 times per label, taking care of loading each labeled ladder alternatively on the right and on the left of the samples.

### 2.2.7. Statistical procedures and tools

To study the marker-trait associations, three different statistical tests were performed, depending on the type of variable and the type of marker considered.

The variables, represented by the traits, were divided into three types: quantitative (AUDPC, DTH, PH, BYDV, IF) ordinal (IT), and binary (presence of a major R gene).

The AFLPs were considered as binary factors that take the value 1 when presence of a band and 0 otherwise. For all types of variables, a linear regression of the trait response on the AFLP was performed using the software GGT. The corresponding r, p-value and q-value were used in the correlations study (Table 2.7). The q-value represents the False Discovery Rate (FDR), corresponding to the part of marker-trait associations declared significant whereas no association existed in reality. This q-value is calculated by the software, based on

the distribution of the p-value.

Each SSR was considered as a group of alleles, each allele representing a binary marker (Kraakman et al., 2006). A multiple regression analysis of each SSR on the quantitative variables was performed using the software GENSTAT. A command file was edited in order to perform all the multiple regressions at once, using a loop (see appendix 7). The corresponding $r^2$ and p-values were used in the association study (Table 2.7)

For the qualitative variables (ordinal and binary), as the model used for the multiple regression analysis did not fit correctly, a one-way ANOVA was preferred. For each SSR-trait combination, the mean of the variable for each allele was calculated and compared with the other means. This test was performed by the GGT software. A significant difference of mean between alleles was interpreted as a correlation SSR-trait. The level of significance, represented by the p-value of the correlation was used for the association study (Table 2.7.).

### 2.2.8. Software

The treatment of the SSR fingerprints was done using the software AFLP-QUANTAR PRO. The statistical software GENSTAT was used to perform the multiple regression analyses. The software GGT (Graphical GenoTyping) was used to perform to perform the simple regreesion analyses and the ANOVA, to calculate and plot the Linkage Disequilibrium and, combined with the Software MEGA, to construct a Neighbour Joining circular relation tree of the collection (appendix 6). MAPCHART software was used to give a graphical view of the integrated maps (fig. 2.3.) and the comparison between the results of Kraakman and the results found in this study (appendix 5).

## 2.3. RESULTS

### 2.3.1. Powdery mildew Resistance

#### 2.3.1.1. Virulence spectrum of the two isolates

The virulence/avirulence spectrum of the two isolates was determined using the Pallas differential series. The resistance genes carried by the cultivars are effective when the isolate possesses the corresponding avirulence gene

Table 2.4. Virulence of the two powdery mildew isolates

| Pallas lines | Resistance gene(s) | Common isolate | Danish isolate |
|---|---|---|---|
| Pallas | *Mla*8 | S[1,2] | S |
| P01 | *Mla*1, *Mla*(Al2) | S | S |
| P02 | *Mla*3 | * | S |
| P03 | *Mla*6, *Mla*14 | S | * |
| P04B | *Mla*7, Ml(No3) | S | * |
| P08B | *Mla*9 | S | S |
| P10 | *Mla*12, Ml(Em2) | S | S |
| P11 | *Mla*13, Ml(Ru3) | S | S |
| P16 | *Mlk* | S | S |
| P23 | *MlLa* | S | * |
| P09 | *Mla*10, Ml(Du2) | S | S |
| P12 | *Mla*22 | S | * |
| P13 | *Mla*23 | * | * |
| P19 | Mlp | * | * |
| P20 | *Mla*t | * | * |
| P21 | *Mlg*, Ml(CP) | S | S |
| P22 | *Mla*5 | * | * |
| P24 | Mlh | S | S |
| P17 | *Mlk*1 | S | S |

[1] S = Susceptible, * = Resistant
[2] A line was considered resistant when the infection type score was lower than the "infection type when resistant" given in the table 2.1.

The Common isolate was more virulent than the Danish isolate. The two isolates differed in virulence for *Mla*3, *Mla*6, *Mla*14, *Mla*7, *Ml(No3) Mla*22 and *MlLa*. Moreover, as expected, they were both avirulent to *mlo*, a recessive gene conferring nearly complete resistance against all isolates of the pathogen (no resistance breakdown).

### 2.3.1.2. Powdery mildew reaction in the collection

Table 2.5. Number of cultivars of the collection per infection type observed

| | Infection Type (IT) observed | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | Missing values |
| Common Isolate I1 | 115 | 1 | 1 | 2 | 15 | 14 |
| Danish Isolate I2 | 117 | 0 | 1 | 2 | 2 | 26 |

During the seedling test, the collection expressed a very high level of resistance to both of the powdery mildew isolates. For the common isolate, 86 % of the studied cultivars did not show any sign of infection for the 4 replications. For the Danish isolate, only 5 cultivars showed signs of infection out of 124 during the 2 replications performed. As expected from a positive control, Manchuria showed a very high level of infection with an infection type of 4 for both isolates. However, for comparable quantities of fresh inocula, the common isolate

always showed much more colony formation than the Danish isolate (at least three times more) on the susceptible cultivars.

#### 2.3.1.3. Major resistance genes in the collection

The review revealed that all of the 99 cultivars of the collection found in the literature carried one or several major R genes for resistance to powdery mildew. For the remaining 49 cultivars, no description of R genes was found in the different sources.

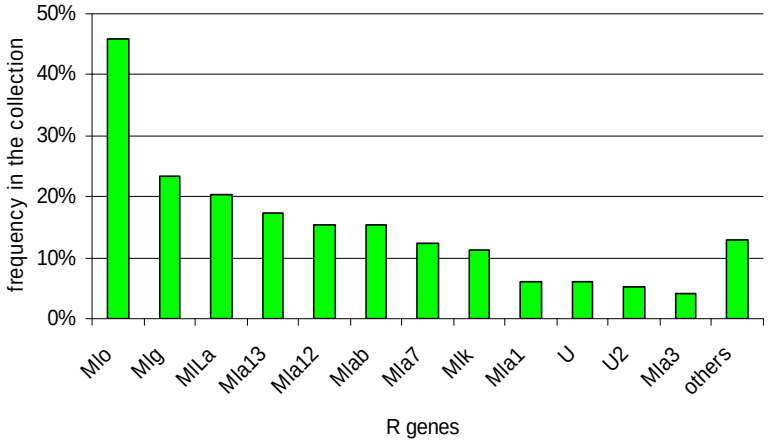



Fig. 2.1. Frequency of Resistance genes in the collection.
"Others" are the R genes showing less than 2% frequency: Ml(lm9), Ml(st), *Mla*4, Ml(Ru3), Ty, Ml(Ra), Ml(hu4), No and Ml(tu2)

Each cultivar had been reported to carry from 1 (Vintage: *Mla*1) to 5 (Optic: *Mlo*, *Mlg*, *MlLa*, *Mla*12 and *Ml(ab)*) major R genes. No Universal Suscept' was found. In the 99 described cultivars, the three most represented R genes were *Mlo* (46%), *Mlg* (24%) and *MlLa* (20%).

The 46 % of mlo cultivars is in agreement with a resistance survey where it was reported to be present in half of the varieties of the Western Europe germplasm (Hovmoller et al., 2000). Concerning *MlLa*, it was rarely found alone in the varieties of the collection. *MlLa* is R gene that confers only intermediate reaction type (IT = 2 or 3) (Hilbers et al., 1992), Therefore, it is usually combined with other R genes in breeding programs. 'Vada' is the only cultivar which is reported to carry *MlLa* alone. It showed susceptibility (IT=4) to I1 and intermediate resistance (IT=3) to I2. This result is in agreement with the one observed in the Pallas differential for the isogenic line carrying only *MlLa* (Table 2.4). As for *MlLa*, *Mlg* was never found alone in the varieties of the collection, probably to increase the resistance durability (gene pyramiding).

Because of their high frequency in the collection (more than 20%). Those three R genes were used as additional parameters for the statistical analysis of associations: They were

considered as binary variables, taking the value 1 when the R gene was reported the cultivar and 0 otherwise, and were named '*mlo presence*', '*Mlg presence*' and '*MlLa presence*'. The other R genes were not included in the association study, as their low frequency and the number of missing values (non-reported cultivars) would decrease dramatically the reliability of those variables.

### 2.3.2. The SSR markers

The 27 selected SSRs were all run on the complete set of DNA. In case the results were not satisfying (no or weak bands, 'wavy' gels…), the study was repeated. Depending on the origin of problem, the whole procedure was done again (PCR and Li-Cor gel) or only the fingerprint (Li-Cor gel). Adaptation of the PCR profile (variation of the temperature and duration of the steps), variations of the quantity of material loaded on the Poly-Acrylamide gel (from 0.4 to 1 µL), use of different PCR machines or DNA sequencer, were the main modification applied to reach a satisfying result.

In the end, out of the 27 selected markers, 3 markers did not produce any valuable results (no fingerprint) even after 3 attempts, out of the 24 markers effectively scored, 3 markers did not show enough polymorphism. The 21 remaining SSRs were integrated in the map and used for the association study. The number of alleles for those SSRs was varying from 2 (HvHVA1, Bmag0222…) to 10 (EBmac0603). Some examples of fingerprints are presented in appendix 4.

### 2.3.3. Positioning of the unmapped markers in the new integrated map

From the 113 unmapped markers by Kraakman, 19 were positioned in the new integrated map (Fig. 2.3) using test for linkage disequilibrium between the AFLP markers performed by the software GGT. A Linkage Disequilibrium plot (LD plaot) was produced (Fig 2.2a, b) and analyzed. A LD plot gives a graphical representation of the level of LD observed for all pairs of markers. Its analysis allowed repositioning some markers to a more suitable position.

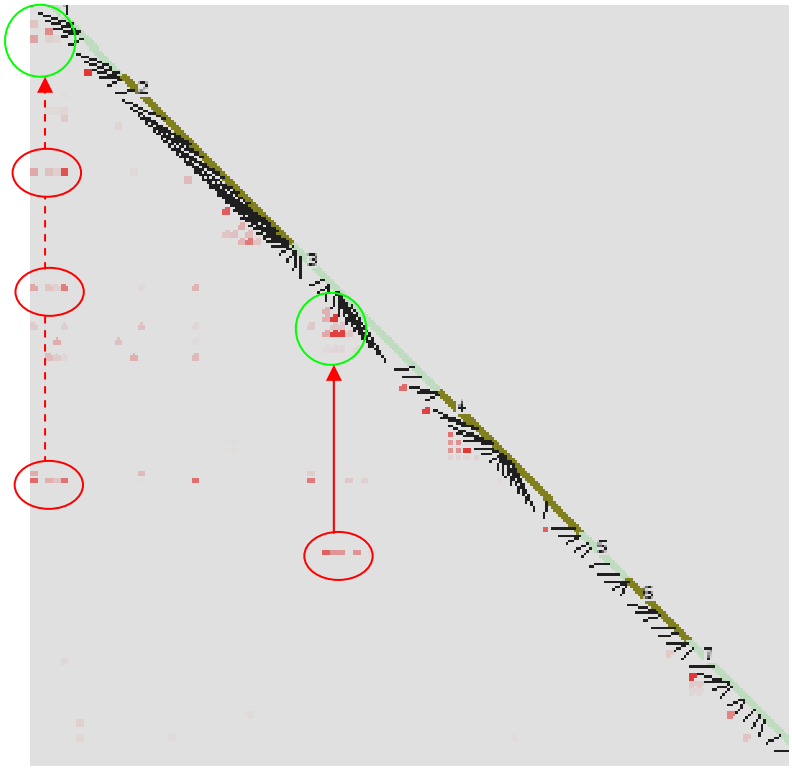


Fig. 2.2a. LD plot of the 113 AFLPs before repositioning of 4 markers.
The chromosomes are aligned on the diagonal with their corresponding markers. The level of association between markers is represented by a square of color at the intersection of the coordinates representing the position of each marker. The colours go from white (no correlation, to red (high correlation). The 'clusters' observed near the chromosomes represent locations were neighboring markers are closely linked to each other.
The red circles highlight the location of 4 markers that show significant association with groups of markers located on completely different chromosome (green circle), suggesting that those markers are more likely to be located in this group than on their present location. Moreover, the three markers showing correlation with markers on chromosome 1 also show correlation between each others (dash red line).

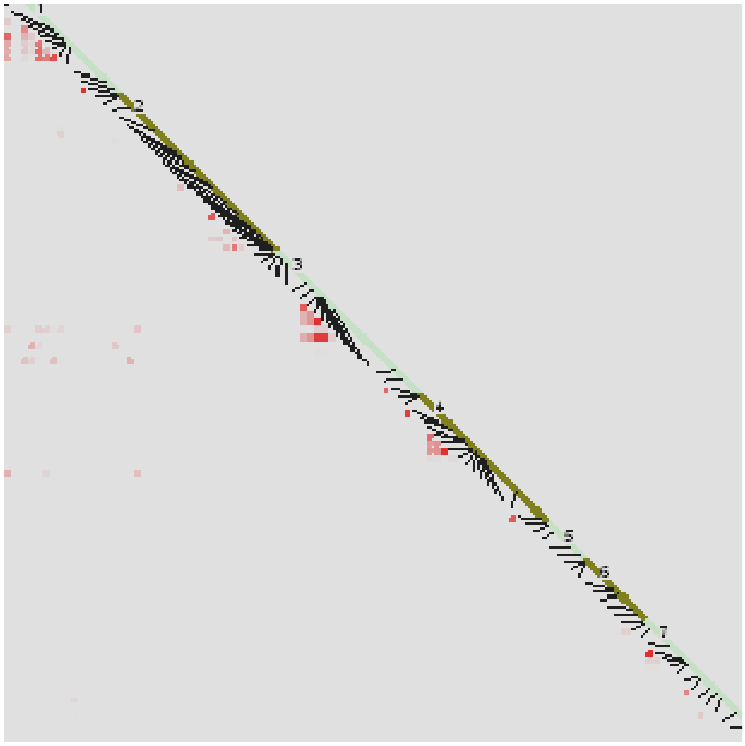


Fig 2.2b. LD plot of the 113 AFLPs after repositioning of the 4 markers.
The new LD plot is of better quality after repositioning of the 4 markers. It shows more correlations between neighboring markers (red spots and clusters near the diagonal) and less correlation between distant markers (red spots far from the diagonal).

After running the LD analysis, 4 markers had an incoherent pattern, showing high correlation with markers linked on a different chromosome than the one where they were mapped in the barley consensus map and three of them were correlated whereas they were mapped in three different chromosomes (dash line). The fact that those markers were linked

to several markers in the same region was sufficient not to consider their position in the consensus map. Therefore, the four markers were moved to chromosome 1 (3 markers) and chromosome 3 (1 marker) where they were assigned a position using the level of correlation they were sharing with markers in those regions. Those misplacements are probably due to the fact that unmapped markers were positioned on the basis of their name and position (see 2.2.5.2), but this method is not perfect and similar name can refer to different markers, generating mistakes. The LD plot analysis allowed correcting some of those mistakes.

### 2.3.4. The new integrated map

Table 2.6. Comparison of markers and gaps of the two maps

| Markers | Kraakman's map | New integrated map |
|---|---|---|
| AFLP mapped | 128 | 99[1] |
| AFLP unmapped | 113 | 0 |
| SSR | 11 | 32[2] |
| Density (marker per cM)[3] | 0.132 | 0.125 |
| Gaps | 13 | 11 |
| <10 cM | 6 | 7 |
| 10 to 20 cM | 3 | 3 |
| >20 cM | 4 | 1 |
| Biggest gap (chromosome) | 58 cM (7H) | 22 cM (7H) |
| Total length of unmapped regions | 207 cM | 95 cM |
| Percentage of the genome unmapped[4] | 19.7 % | 9.0 % |

[1] 80 AFLPS mapped in Kraakman et al. (2006) and 19 unmapped markers repositioned
[2] The 11 SSRs from Kraakman et al. (2006) and 21 SSRs added
[3] Total number of marker divided by the size of the barley genome (1052cM)
[4] Total length of unmapped regions divided by the size of the barley genome (1052cM)

The new integrated map contained 131 markers (fig.2.3): The 80 AFLPs and the 11 SSRs mapped in Kraakman et al. (2006) (in black on fig.2.3), the 19 of the unmapped AFLPs repositioned (in green) and the 21 SSRs added during this study (in red). Compared to Kraakman's map, the new integrate map contained less and smaller gaps. It showed a better coverage even if it is composed of a similar number of markers (Table 2.6). Despite this relatively good general coverage, big differences were still observed between chromosomes: the chromosome 2H contained 25 markers whereas chromosome 1H contained only 8 markers.

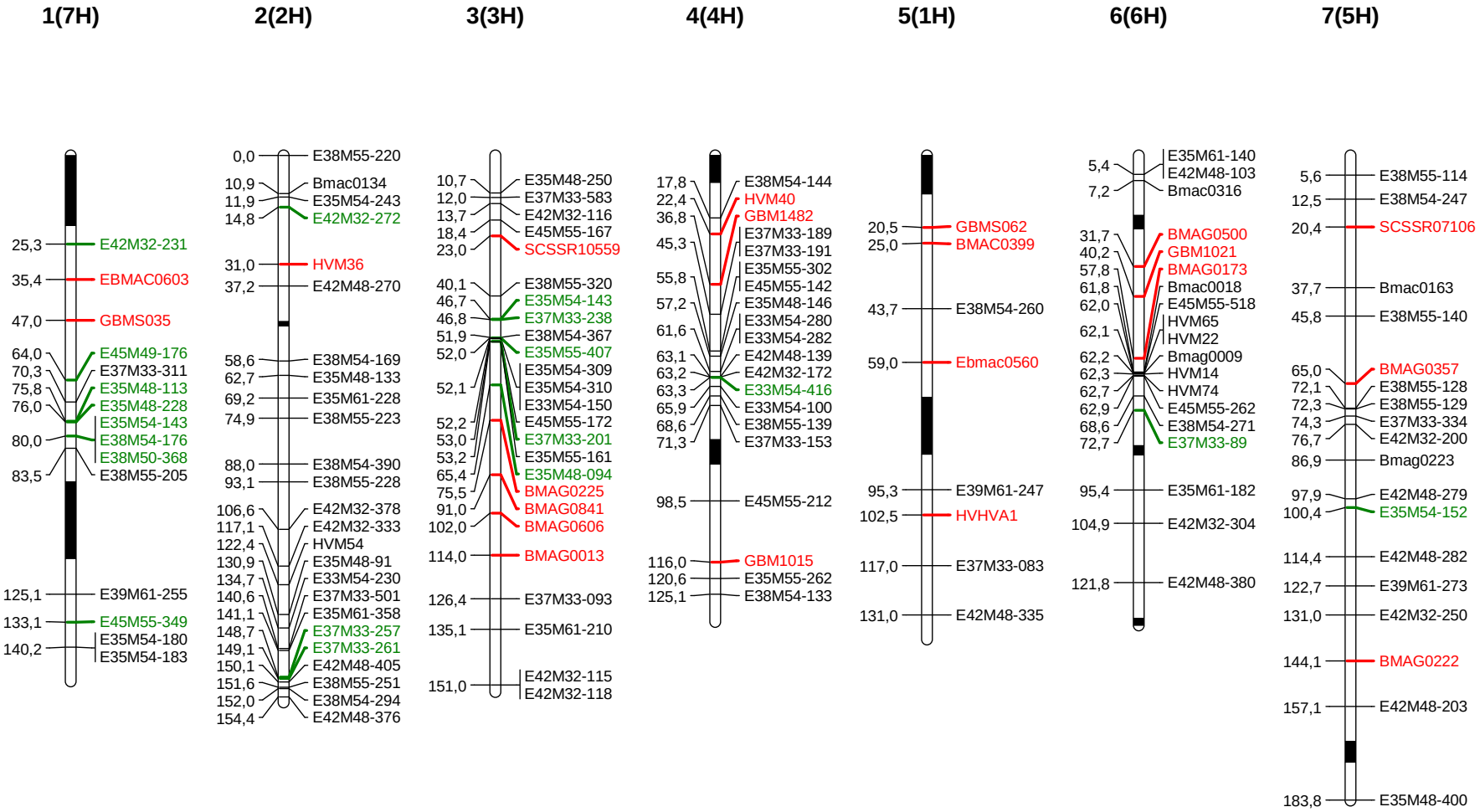



Fig.2.3. Integrated map showing the genetic position of the 99 AFLP markers and 32 SSR markers used for the association study. The black segments correspond to the "gaps", the areas where to consecutive markers were separate by more than 20 cM, assuming that a linkage disequilibrium distance of 10 cM (Kraakman et al. 2004).
In green, the AFLP markers unmapped in Kraakman et. Al, 2006.
In red, the SSR markers that were added for this study.

The chromosome lengths are quite comparable between the two maps. Chromosomes 3 and 6 have similar sizes in both maps. Chromosomes 2, 4 and 7 are longer in the new map. Chromosomes 1 and 5 are shorter. Differences of distances between couples of common markers are generally low and switches in the marker order are limited to the markers positioned near the centromere where it is usually observed (Karakousis et al., 2003). A good example of those switches at the centromeric region is observed on the chromosome 7 (appendix 5). However, some radical differences in the positioning of several markers are observed (see appendix 5). It is mainly the case for the SSR markers. This result is not surprising as SSR have been reported to be difficult to align and are generally used in combination with other types of markers (AFLP, RFLP) to create integrated maps (Karakousis et al., 2003). The position of those SSRs in the barley consensus map is considered to be more reliable as the marker positions were derived from more mapping populations. The position Bmac0134 in the consensus map, for example, was derived from four mapping populations: L94 x Vada, Igri x Franka, Oregon Wolf Barley and SusPtrit x Cebada Capa.

### 2.3.5. Associations marker-trait

The results of the statistical tests for associations are presented in table 2.7. A comparison of the two maps, chromosome by chromosome, is given in appendix 5.

#### 2.3.5.1. Traits from the previous study

The statistical test for association was performed between the markers of the new integrated map and the data that were collected by Kraakman (see 2.1.2 and (Kraakman et al., 2006) for the details of those traits). In Kraakman's study, several correlations were discarded despite their statistical significance. Some correlations were not recorded when they involved a single marker and a trait in a region where no QTL had been reported before. Those correlations were also discarded when they were observed in the new integrated map (same marker, same trait, no reported QTL in the region and no correlation with other markers in the same region).

Table 2.7 All marker-trait associations found in the barley collection where at least one association (in bold) had a false discovery rate lower than 5% (q<0.05)

| | | | AUDPC>6[5] | | IT | | LP | | BYDV | | DTH | PH | | IF1 | | IT1 | | IF1 susc. | IF2 | | IT2 | | Mlo | | MlLa | | Mlg | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Marker [1] | chr | pos[2] | Corr.[3] | *p* [4] | Corr. | *p* | Corr. | *p* | Corr. | *p* | Corr. *p* | Corr. | *p* | Corr. | *p* | Corr. | *p* | Corr. *p* | Corr. | *p* | Corr. | *p* | Corr. | *p* | Corr. | *p* | Corr. | *p* |
| E42M32-231 | 1 | 25.313 | 0.11 | | -0.03 | | 0.09 | | 0.05 | | 0.00 | **-0.27** | * | 0.05 | | 0.02 | | -0.56 | 0.20 | | 0.18 | | -0.26 | | 0.13 | | 0.11 | |
| **EBmac0603** | 1 | 35.390 | 0.00 | | | | 0.00 | | 0.02 | | 0.02 | **0.09** | * | **0.08** | * | | | 0.50 | 0.00 | | | | | | | | | |
| *E45M49-176* | 1 | 63.976 | 0 | | -0.10 | | 0.10 | | **0.39** | *** | -0.09 | -0.10 | | -0.03 | | 0.01 | | 0.03 | -0.08 | | -0.04 | | -0.05 | | -0.14 | | 0.07 | |
| *E35M48-113* | 1 | 75.822 | 0.01 | | -0.08 | | -0.02 | | 0.08 | | -0.11 | **0.33** | *** | 0.04 | | 0.11 | | 0.41 | -0.09 | | -0.09 | | -0.04 | | -0.01 | | -0.02 | |
| *E35M48-228* | 1 | 76.000 | -0.1 | | 0.11 | | 0.06 | | 0.02 | | -0.11 | **0.29** | ** | 0.05 | | 0.14 | | 0.37 | 0.06 | | -0.07 | | 0.18 | | -0.11 | | -0.10 | |
| E39M61-255 | 1 | 125.104 | -0.11 | | **0.75** | **** | -0.05 | | 0.04 | | 0.10 | -0.04 | | 0.14 | | 0.13 | | 0.11 | 0.05 | | 0.04 | | -0.12 | | -0.04 | | 0.21 | |
| *E45M55-349* | 1 | 133.136 | -0.02 | | 0.15 | | 0.10 | | 0.02 | | -0.02 | -0.12 | | 0.23 | | 0.17 | | -0.46 | **0.35** | ** | **0.40** | **** | -0.02 | | 0.11 | | 0.10 | |
| E35M54-180 | 1 | 140.172 | -0.01 | | 0.05 | | 0.01 | | **0.28** | ** | 0.16 | -0.12 | | 0.00 | | 0.00 | | -0.01 | 0.09 | | 0.08 | | -0.07 | | -0.10 | | 0.07 | |
| E35M54-183 | 1 | 140.172 | -0.01 | | -0.05 | | -0.01 | | **-0.28** | ** | -0.16 | 0.12 | | 0.00 | | 0.00 | | -0.01 | -0.08 | | -0.07 | | 0.07 | | 0.10 | | -0.07 | |
| Bmac0134 | 2 | 10.867 | 0.00 | | | * | 0.03 | | 0.01 | | 0.02 | 0.00 | | 0.00 | | | | 0.00 | **0.25** | *** | | *** | | | | | | |
| **HVM036** | 2 | 30.970 | 0.00 | | | | 0.02 | | 0.00 | | 0.01 | 0.00 | | 0.00 | | | | **0.38** * | 0.00 | | | | | | | | | |
| E35M61-228 | 2 | 69.163 | 0.23 | | 0.06 | | -0.31 | * | **0.47** | **** | -0.02 | 0.05 | | -0.18 | | -0.12 | | -0.29 | -0.08 | | -0.19 | | 0.02 | | -0.08 | | 0.16 | |
| HVM54 | 2 | 122.406 | 0.00 | | | | 0.05 | | **0.41** | *** | 0.03 | 0.00 | | 0.02 | | | | **0.45** * | 0.00 | | | | | | | | | |
| E35M48-91 | 2 | 130.913 | 0.04 | | -0.01 | | 0.19 | | 0.16 | | -0.12 | -0.03 | | 0.12 | | 0.06 | | -0.51 | **0.30** | ** | 0.12 | | 0.07 | | -0.08 | | -0.09 | |
| *E37M33-257* | 2 | 148.744 | 0.06 | | 0.09 | | -0.07 | | **-0.31** | ** | -0.10 | 0.13 | | -0.02 | | -0.01 | | 0.34 | -0.01 | | -0.06 | | **0.31** | * | -0.28 | * | -0.18 | |
| *E37M33-261* | 2 | 149.129 | 0.06 | | -0.09 | | 0.00 | | **0.23** | * | 0.03 | -0.06 | | 0.02 | | -0.02 | | 0.34 | 0.00 | | 0.04 | | **-0.29** | * | 0.25 | | 0.14 | |
| E42M48-405 | 2 | 150.062 | 0.08 | | 0.19 | | 0.25 | * | 0.15 | | 0.00 | -0.11 | | **0.41** | **** | **0.36** | *** | -0.71 | 0.22 | | 0.24 | * | -0.17 | | **0.35** | ** | 0.08 | |
| E38M55-251 | 2 | 151.561 | 0.05 | | 0.22 | * | **0.34** | ** | -0.06 | | -0.03 | -0.10 | | **0.28** | * | 0.25 | * | -0.67 | 0.24 | | 0.27 | * | -0.01 | | 0.14 | | -0.01 | |
| E38M54-294 | 2 | 151.984 | -0.1 | | **-0.33** | *** | -0.01 | | 0.10 | | 0.02 | 0.15 | | **-0.29** | ** | -0.23 | * | 0.62 | -0.20 | | -0.20 | | 0.02 | | -0.02 | | -0.07 | |
| E42M48-376 | 2 | 154.422 | 0.05 | | **0.31** | ** | 0.29 | * | -0.03 | | -0.08 | -0.15 | | **0.24** | * | 0.19 | | -0.71 | 0.07 | | 0.21 | | -0.03 | | 0.25 | | 0.09 | |
| *E35M48-94* | 3 | 65.379 | -0.02 | | -0.08 | | -0.08 | | -0.01 | | -0.14 | **0.28** | ** | -0.04 | | 0.09 | | 0.63 | 0.01 | | -0.09 | | -0.01 | | -0.07 | | -0.02 | |
| **Bmag0225** | 3 | 75.540 | 0.00 | | | | **0.09** | * | 0.00 | | 0.00 | 0.00 | | 0.01 | | | | 0.00 | 0.00 | | | | | | | | | |
| E37M33-93 | 3 | 126.421 | -0.07 | | -0.01 | | -0.07 | | 0.17 | | 0.00 | -0.15 | | **0.28** | * | 0.15 | | -0.68 | **0.35** | *** | **0.46** | **** | -0.13 | | 0.23 | | 0.08 | |
| **HVM040** | 4 | 22.400 | 0.00 | | | | 0.03 | | **0.08** | ** | 0.01 | 0.00 | | 0.00 | | | | 0.00 | 0.00 | | | | | | | | | |
| **GBM1482** | 4 | 36.810 | 0.00 | | | | 0.00 | | 0.00 | | 0.00 | 0.00 | | 0.00 | | | | 0.00 | **0.09** | *** | | ** | | | | | | * |
| E35M55-302 | 4 | 55.763 | 0.06 | | -0.02 | | -0.06 | | **0.25** | * | -0.08 | -0.07 | | -0.19 | | -0.14 | | -0.27 | 0.03 | | 0.04 | | 0.18 | | -0.16 | | -0.12 | |
| E45M55-142 | 4 | 55.763 | -0.15 | | -0.10 | | 0.07 | | 0.02 | | -0.14 | 0.11 | | -0.10 | | -0.10 | | 0.06 | -0.02 | | -0.11 | | **0.37** | ** | -0.13 | | -0.19 | |
| E33M54-280 | 4 | 61.632 | -0.13 | | -0.04 | | 0.18 | | -0.06 | | -0.23 | 0.21 | | -0.16 | | -0.17 | | n.c | 0.05 | | -0.09 | | **0.59** | **** | -0.27 | | -0.26 | |
| E33M54-282 | 4 | 61.632 | -0.13 | | 0.04 | | -0.17 | | 0.06 | | 0.23 | -0.21 | | 0.16 | | 0.17 | | n.c | -0.05 | | 0.09 | | **-0.60** | **** | 0.28 | | 0.27 | |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E42M48-139 | 4 | 63.099 | -0.14 | | 0.15 | | -0.04 | | 0.01 | | -0.19 | | -0.04 | | -0.11 | | -0.11 | | n.c | | -0.06 | | -0.06 | | **0.33** | ** | -0.15 | | -0.16 | |
| *E33M54-416* | 4 | 63.327 | 0.26 | | -0.02 | | -0.23 | | **0.29** | * | 0.10 | | -0.06 | | -0.17 | | -0.17 | | -0.06 | | 0.02 | | -0.06 | | 0.11 | | -0.14 | | 0.04 | |
| E33M54-100 | 4 | 65.920 | -0.1 | | -0.06 | | -0.03 | | -0.11 | | 0.11 | | -0.01 | | **-0.28** | * | -0.19 | | -0.5 | | -0.10 | | -0.24 | * | 0.17 | | -0.19 | | -0.33 | ** |
| E38M55-139 | 4 | 68.628 | -0.09 | | -0.03 | | -0.02 | | **0.25** | * | -0.02 | | -0.04 | | 0.05 | | 0.02 | | 0.08 | | 0.13 | | 0.16 | | **-0.31** | * | 0.27 | * | 0.31 | * |
| E45M55-212 | 4 | 98.496 | 0.28 | | 0.06 | | 0.05 | | **-0.25** | * | 0.11 | | 0.04 | | **0.34** | *** | **0.30** | ** | -0.37 | | 0.13 | | **0.32** | ** | **-0.31** | * | **0.32** | * | 0.02 | |
| **GBMS062** | 5 | 20.500 | 0.00 | | | | 0.00 | | 0.03 | | 0.00 | | **0.05** | * | 0.00 | | | | 0.13 | | 0.00 | | | | | | | | | |
| **bmac0399** | 5 | 25.000 | 0.00 | | | | 0.03 | | **0.05** | * | 0.01 | | 0.00 | | 0.04 | | | | 0.11 | | **0.05** | * | | * | | | | | | |
| E38M54-260 | 5 | 43.686 | -0.08 | | **0.27** | * | 0.03 | | -0.02 | | -0.13 | | 0.11 | | 0.18 | | 0.07 | | 0.58 | | 0.11 | | 0.09 | | -0.13 | | 0.06 | | 0.20 | |
| E39M61-247 | 5 | 95.263 | 0.09 | | 0.12 | | 0.11 | | **-0.24** | * | 0.00 | | -0.13 | | **0.27** | * | 0.14 | | -0.61 | | 0.18 | | 0.13 | | -0.01 | | 0.22 | | 0.07 | |
| **HvhVA1** | 5 | 102.490 | 0.00 | | | * | 0.00 | | 0.00 | | 0.00 | | 0.01 | | **0.03** | * | | | 0.05 | | 0.00 | | | | | | | | | |
| Bmac0316 | 6 | 7.155 | 0.00 | | | * | 0.00 | | 0.03 | | 0.00 | | 0.00 | | 0.00 | | | | 0.00 | | 0.01 | | | | | | | | | |
| **Bmag0500** | 6 | 31.650 | **0.25** | *** | | ** | 0.05 | | 0.06 | | **0.09** | * | 0.04 | | 0.01 | | | | 0.47 | | **0.10** | * | | ** | | | | | | |
| **GBM1021** | 6 | 40.170 | **0.06** | * | | | 0.02 | | **0.04** | * | 0.00 | | 0.00 | | 0.02 | | | | 0.02 | | 0.00 | | | | | | | | | |
| Bmac0018 | 6 | 61.792 | 0.02 | | | | 0.00 | | 0.01 | | 0.00 | | **0.03** | * | 0.00 | | | | 0.00 | | 0.00 | | | | | | | | | |
| HVM65 | 6 | 62.110 | 0.01 | | | | 0.01 | | 0.00 | | 0.00 | | 0.04 | | **0.07** | ** | | | 0.00 | | 0.00 | | | | | | | | | |
| HVM22 | 6 | 62.143 | 0.01 | | | | 0.00 | | 0.01 | | 0.00 | | **0.04** | * | 0.00 | | | | 0.00 | | 0.00 | | | | | | | | | |
| **Bmag0009** | 6 | 62.211 | 0.01 | | | | 0.00 | | 0.00 | | 0.00 | | 0.02 | | 0.00 | | | | 0.00 | | 0.00 | | | | | | | | | |
| HVM14 | 6 | 62.278 | 0.01 | | | | 0.01 | | 0.01 | | 0.00 | | **0.02** | * | 0.00 | | | | 0.00 | | 0.00 | | | | | | | | | |
| HVM74 | 6 | 62.658 | 0.01 | | | * | 0.00 | | 0.01 | | 0.00 | | **0.03** | * | 0.00 | | | | 0.00 | | 0.00 | | | | | | | | | |
| Bmac0163 | 7 | 37.735 | 0.00 | | | | 0.00 | | **0.05** | ** | 0.00 | | **0.04** | * | 0.00 | | | | 0.00 | | 0.00 | | | | | | | | | |
| **Bmag0357** | 7 | 65.040 | 0.00 | | | * | 0.02 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | * | | 0.28 | | 0.00 | | | | | | | | | |
| E38M55-128 | 7 | 72.059 | **0.31** | * | 0.06 | | -0.01 | | 0.18 | | -0.04 | | -0.12 | | 0.15 | | 0.12 | | -0.15 | | 0.09 | | 0.09 | | -0.16 | | 0.00 | | 0.03 | |
| E38M55-129 | 7 | 72.292 | **0.31** | * | -0.06 | | 0.01 | | -0.18 | | 0.04 | | 0.12 | | -0.15 | | -0.12 | | -0.15 | | -0.09 | | -0.09 | | 0.16 | | 0.00 | | -0.03 | |
| E42M32-200 | 7 | 76.744 | **0.35** | ** | 0.13 | | 0.26 | * | 0.14 | | 0.04 | | 0.00 | | 0.20 | | 0.13 | | -0.39 | | 0.07 | | 0.08 | | -0.11 | | 0.16 | | 0.01 | |
| Bmag0223 | 7 | 86.880 | 0.00 | | | | 0.00 | | 0.01 | | **0.06** | * | **0.01** | ** | 0.02 | | | | **0.50** | * | 0.00 | | | | | | | | | |
| E42M32-250 | 7 | 130.999 | 0.01 | | 0.12 | | 0.08 | | 0.04 | | 0.17 | | -0.17 | | **0.29** | ** | 0.17 | | -0.56 | | 0.23 | | 0.23 | | **-0.29** | * | **0.36** | ** | 0.13 | |
| **Bmag0222** | 7 | 144.140 | 0.00 | | | | 0.00 | | 0.00 | | 0.00 | | 0.01 | | 0.04 | | | | **0.49** | ** | 0.02 | | | * | | | | | | |
| E42M48-203 | 7 | 157.148 | 0.02 | | 0.01 | | 0.09 | | -0.01 | | -0.07 | | -0.09 | | **0.26** | * | 0.15 | | -0.67 | | 0.22 | | **0.36** | ** | **-0.27** | * | **0.37** | ** | 0.27 | |

[1] Markers, regular : AFLPs and SSR markers used in the study of Kraakman et al. (2006), **bold**: SSRs added to the map. *Italic*, AFLPs unmapped in Kraakman et. al, 2006.
[2] Marker map positions were based on the new integrated map (Fig.2.5.)
[3] Indices of correlation: For the SSRs, the significance of correlation is shown as *p*-value and $r^2$ (multiple linear regression) or *p*-value only (one way ANOVA).
For the AFLPs, the significance of correlation is shown as *p*-value and r (simple linear regression).
[4] Significance of p*, **, ***, $p<0.05$, 0.01, 0.001, respectively. Correlation in bold are statistically significant in the corresponding statistical analysis.
[5] For details about the traits, see 2.2.4.1 (powdery mildew) and Kraakman et. al, 2006 (other traits).

The comparisons between the results of Kraakman et al. (2006) and the present analysis were reported trait by trait (Table 2.8.a, b and c).

Table 2.8a. Comparison of the QTLs for Leaf rust resistance found in the two studies

| QTLs found in Kraakman's study | | | | | QTLs found in this study | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Name | Chr. | Pos. (cM) | Markers[1] | Reported[2] | Name | Chr. | Pos. (cM) | Markers | Reported |
| *Rph3* | 1 | 151 | 1 | Yes | *Rph3* | 1 | 125 | 1 | Yes |
| IT1 | 2 | 137-142 | 2 | No | IT1 | 2 | 151-152 | 2 | No |
| IT? | | unmapped | 1 | | | | | | |
| | | | | | IT2 | 6 | 7-32 | 2 | No |
| | | | | | IT3 | 7 | 65 | 1 | No |
| AUDPC1 | 7 | 69 | 4 | No | AUDPC1 | 7 | 72 | 3 | No |
| | | | | | AUDPC2 | 6 | 32-40 | 2 | No |
| RLP1 | 2 | 49 | 1 | Yes | RLP1 | 2 | 69 | 1 | Yes |
| RLP2 | 2 | 89 | 1 | Yes | | | | | |
| *Rphq2* | 2 | 136-142 | 3 | Yes | *Rphq2* | 2 | 150-154 | 3 | Yes |
| RLP4 | 3 | 56-76 | 3 | No | RLP4 | 3 | 75 | 1 | No |
| RLP? | | unmapped | 9 | | | | | | |

[1] Number of markers found correlated to this QTL or, in case of the unmapped QTLs, number of unmapped markers related to the trait

[2] Presence of a reported QTL in the same region

The infection type IT is an indicator of the presence of a major resistance gene (like *Rph3*). A low IT corresponds to an incompatible interaction between the pathogen and the plant due to the presence of a gene-for-gene interaction. On the other hand, RLP and AUDPC are quantitative measures related to partial resistance. AUDPC was measured on cultivars showing high IT, meaning the cultivars that do not carry a major resistance gene. This allows not confounding the effect of major R genes, to be able to focus on partial resistance (Kraakman et al., 2006). The same method was applied in this study for partial resistance to barley powdery mildew with the parameter 'IF susc' (see 2.3.5.2).

Concerning the correlations of markers with IT, the major R gene *Rph3* and the QTL found on chromosome 2, found by Kraakman (IT1), were also positioned in the new integrated map at similar positions. Additionally, a correlation between the SSR Bmag0357 and IT was found on chromosome 7 (IT3), near from a position where Kraakman et Al. found a correlation with AUDPC (also retrieved in this study AUDPC1). It suggests the presence of a non reported QTL for partial resistance to Leaf Rust and a major R gene in the same region. A similar co-localization of AUDPC (AUDPC2) and IT (IT2) was observed on the top of chromosome 6. This region corresponds to a gap in Kraakman's map that appeared also in the new integrated map and was filled up with SSR markers.

Concerning RLP, two of the QTL reported by Kraakman et Al. on chromosome 2 were found back (*Rphq2* and RLP1). On chromosome 3, RLP was correlated with Bmag0225. It

suggests the presence of a QTL in this region as reported by Kraakman who found that RLP was correlated with three markers in this region (but those markers were not conserved in the new map). None of the 9 unmapped markers correlated to RLP in Kraakman's study were positioned in the new map.

Table 2.8b. Comparison of the QTLs for BYDV tolerance found in the two studies

| QTLs found in Kraakman's study | | | | | QTLs found in this study | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Name | Chr. | Pos. (cM) | Markers[1] | Reported[2] | Name | Chr. | Pos. (cM) | Markers | Reported |
| BYD1 | 1 | 9 | 1 | Yes | | | | | |
| BYD2 | 1 | 163 | 1 | Yes | BYD2 | 1 | 140 | 2 | Yes |
| BYD3 | 2 | 49 | 1 | Yes | BYD3 | 2 | 69 | 1 | Yes |
| BYD4 | 2 | 95 | 1 | No | BYD4 | 2 | 122 | 1 | No |
| BYD5 | 4 | 0 | 1 | No | BYD5 | 4 | 22 | 1 | No |
| BYD6 | 4 | 45 | 1 | No | BYD6 | 4 | 63-67 | 2 | No |
| BYD? | | unmapped | 6 | | BYD7 | 1 | 64 | 1 | No |
| | | | | | BYD8 | 2 | 149 | 2 | Yes |

[1] Number of markers found correlated to this QTL or, in case of the unmapped QTLs, number of unmapped markers related to the trait
[2] presence of a reported QTL in the same region

Kraakman found many markers associated with the tolerance to BYDV. But, the QTL they found on the top the chromosome 1 (BYD1) was not confirmed as it is located in a gap of the new map. BYD2 was present in the new map and a new one, BYD7 was found correlated with an unmapped markers repositioned at 64 cM. On chromosome 2, the two QTL from Kraakman (BYD3 and BYD4) were positioned in the new map and a new QTL (BYD8) was added from a correlation with two unmapped markers positioned in the same region. A QTL for tolerance to BYDV (related to yield reduction) has been reported in this region (Friedt et al., 2003). Additional correlations were found with SSRs on chromosome 5 (Bmac0399) and chromosome 6 (GBM1021), but they were not reported in the literature and the significance of correlation was low. Therefore, it was not possible to insure that those associations between BYDV and isolated SSRs were, indeed, due to the presence of QTLs in those regions.

Table 2.8c. Comparison of the QTLs for Days To Heading and Plant Height found in the two studies

| QTLs found in Kraakman's study | | | | | QTLs found in this study | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Name | Chr. | Pos. (cM) | Markers[1] | Reported[2] | Name | Chr. | Pos. (cM) | Markers | Reported |
| DTH1 | 7 | 32 | 1 | Yes | | | | | |
| DTH? | | unmapped | | | | | | | |
| PH1 | 1 | 24 | 1 | Yes | PH1 | 1 | 25-35 | 2 | Yes |
| PH2 | 1 | 106 | 1 | Yes | PH2 | 1 | 75-76 | 2 | Yes |
| PH3 | 7 | 76 | 1 | Yes | PH3 | 7 | 86 | 1 | Yes |
| PH? | | Unmapped | 8 | | PH4 | 3 | 65 | 1 | Yes |

[1] Number of markers found correlated to this QTL or, in case of the unmapped QTLs, number of unmapped markers related to the trait
[2] presence of a reported QTL in the same region

The QTL related to DTH reported by Kraakman on the chromosome 7 (DTH1) was not indicated in the new integrated map as it was located in a region where the density of marker was very low. A weak correlation was found on chromosome 6 with the SSR marker Bmag0500, but no QTL related to DTH has been reported in this region (Kicherer et al., 2000; Marquez-Cedillo et al., 2001).

The three QTL related to PH were confirmed in the new integrated map. Two unmapped markers showing high correlation with PH (E35M48-113 and E35M48-228) were repositioned in a region that seems to be the one where PH2 was mapped by Kraakman. However, it cannot be verified as the marker linked to PH2 in Kraakman study was not conserved in the new integrated map? For the other QTL (PH1), the correlated AFLP was conserved and a SSR added to the same region showed also correlation with PH. Finally, one of the unmapped markers showing correlation with PH (E35M48-94) was positioned in the centromeric region of chromosome 3 where a QTL for PH was reported (Qi et al., 1998).

#### 2.3.5.2. Powdery mildew resistance

The AFLP and SSR markers showed correlation with infection type and infection frequency of the isolates. IFsusc is a measure of the infection frequency for the cultivars showing a compatible interaction with the pathogen (IT=4). Similarly to AUDPC in Kraakman's study, it allows concentrating on the variation in level of partial resistance without confounding with the effect of major genes for resistance (Kraakman et al., 2006). This parameter was calculated only for the Common Isolate (I1) because the number of cultivars showing compatible interaction with the Danish Isolate (I2) was not sufficient.

The significance of correlation (p-value) between the markers and IF1, IT1, IF2, IT2 and IFsusc were plotted against the position of the markers for each of the chromosomes (Fig 2.4.a, b, c, d, e, f and g). The arrows in those plots highlight the regions where the observed correlations were significantly related to the presence of a R gene (associated with IT) and/or a QTL (associated with IF) for powdery mildew resistance.

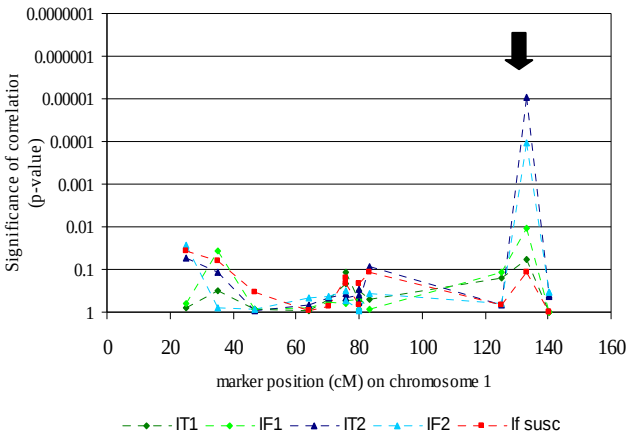



Fig 2.4.a. Association profile of IF and IT for the two isolates of *Blumeria graminis* on chromosome 1. The position of putative QTL or R genes is indicated with an arrow

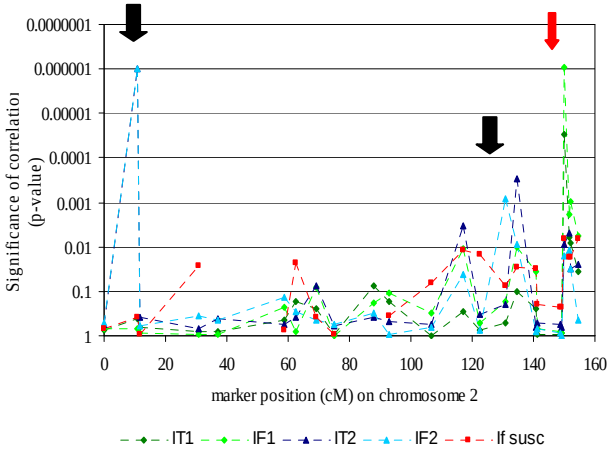



Fig 2.4.b. Association profile of IF and IT for the two isolates of *Blumeria graminis* on chromosome 2. The position of putative QTL is indicated with a black arrow. The red arrow indicates the position of the *MlLa* gene

A peak of correlation for IF2 and IT2 was observed on the long arm of chromosome 1 (130 cM). IF2 and IT2 showed a very significant association with the marker E45M55-349. This marker was not positioned in the previous map. In the literature, *Mlf*, a major gene and a QTL have been reported in this region (Backes et al., 2003). Given that the correlation concerns essentially isolate 2, it is expected that *Mlf* is efficient only against this isolate (incompatible interaction). However, this hypothesis cannot be confirmed as *Mlf* is not represented in the Pallas differential series for the virulence spectrum testing and has not been recorded in the studies used for the R gene review. A QTL for partial resistance have been reported in the same region as *Mlf* (Backes et al., 2003) but it is more likely that the correlations observed here were due to the R gene and not to the presence of a QTL because no significant correlation with IFsusc was observed.

Considering that correlations found between a trait and markers located at less than 20 cM are reflecting the presence a single QTL, two QTLs and one R gene were indicated. On chromosome 2, the high correlation of IF2 around 10 cM indicated the presence of a reported QTL for partial resistance to powdery mildew (Backes et al., 2003). The high correlation with IT2 in the same region was not related to presence of a reported R gene in this region. The presence of a QTL around 120 cM has not been reported in the literature. However, the existence of a significant correlation between IFsusc and 2 markers located in the same region (HVM054 and E42M32-333) is a good indication of the presence of a non reported QTL. Finally, a group of 4 markers located at the end of chromosome 2 (around 150 cM) was found to be correlated with IF1, IT1 and 'MlLa presence'. The variable 'MlLa presence' showed a

very significant correlation with the marker E42M48-405. It is in agreement with the location of *MlLa* given in several studies (Backes et al., 2003; Hilbers et al., 1992; Saghai Maroof et al., 1994). At this location, markers were also correlated with leaf rust reaction (LP and IT). The association of the *MlLa* R gene with QTL for partial resistance to leaf rust (*Rphq2*) has been reported in this region especially with the AFLP marker E38M55-251 (Kraakman et al., 2006). *MlLa* and *Rphq2* are both originating from *Hordeum Laevigatum* (Kraakman et al., 2006) and segregated together in most cases. This genetic link is used in indirect test to check the presence of *Rphq2* by inoculating the plant with a mildew isolate avirulent to *MlLa* (technique performed in the lab of Plant Breeding using I2).

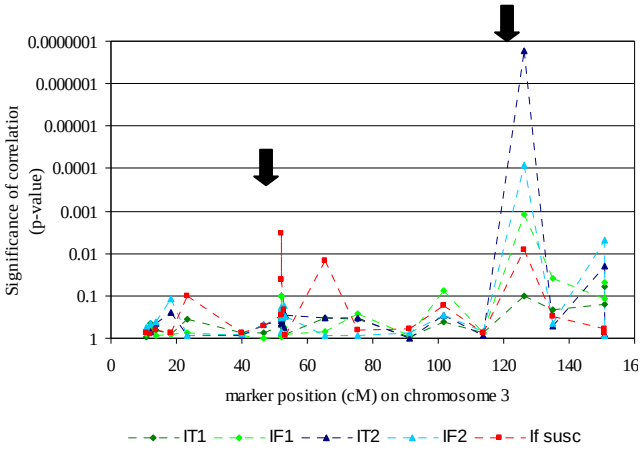



Fig 2.4.c. Association profile of IF and IT for the two isolates of *Blumeria graminis* on chromosome 3. The position of putative QTL or R genes is indicated with an arrow

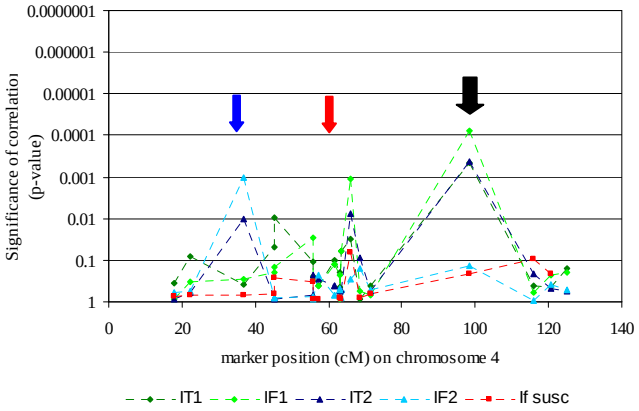



Fig 2.4.d. Association profile of IF and IT for the two isolates of *Blumeria graminis* on chromosome 4. The position of putative QTL is indicated with black arrow, *Mlo* with a red arrow and *Mlg* with a blue arrow

On chromosome 3, two putative QTL were found. Around 60 cM, several markers were found to be significantly correlated to IFsusc This is a region where the QTL Rbgq2 have been mapped (Shtaya et al., 2006). The other correlation found at 125 cM. A QTL was reported in this region in an infection test on detached leaves (Backes et al., 2003).

The association profile of chromosome 4 shows three distinct peaks. Moreover, the variable 'm*lo* presence' showed highly significant correlation with 4 markers at the centromeric region of the chromosome (55 to 60 cM). '*Mlg* presence' was correlated with the SSR GBM1482. The literature confirms the presence of m*lo* and *Mlg* on this chromosome (Chelkowski et al., 2003; Saghai Maroof et al., 1994; Shtaya et al., 2006). The highly significant correlations between IF1, IT1, IF2 and the marker E45M55-212 (98 cM) may indicate the presence of a non-reported QTL. However, additional markers would be needed in this poorly described region to confirm completely this hypothesis.

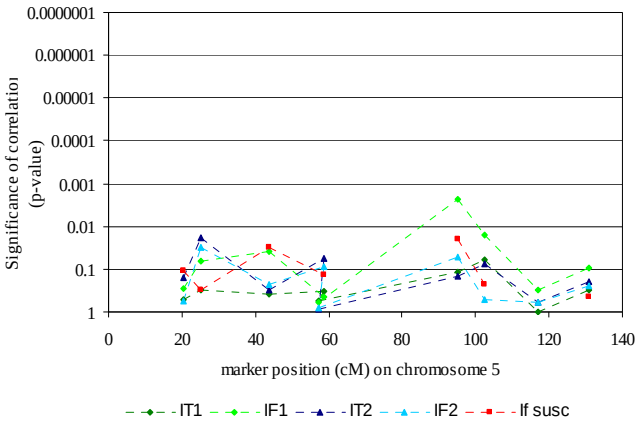



Fig 2.4.e. Association profile of IF and IT for the two isolates of *Blumeria graminis* on chromosome 5

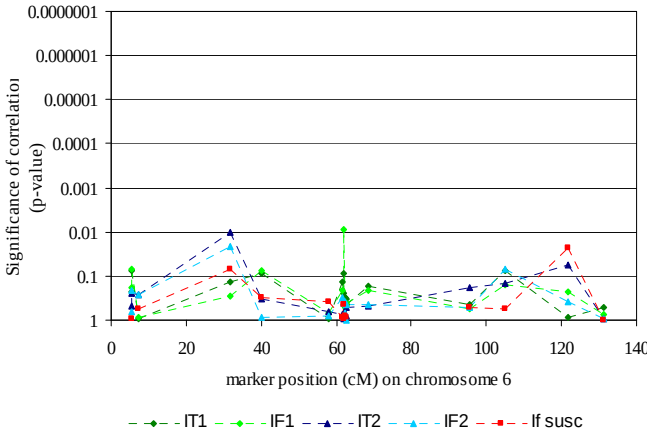
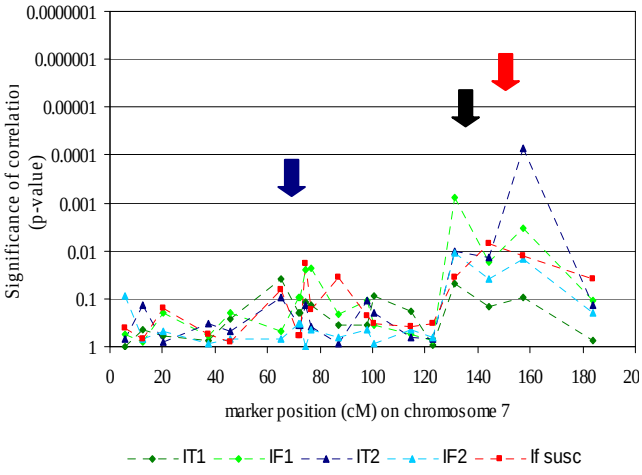



Fig 2.4.f. Association profile of IF and IT the two isolates of *Blumeria graminis* on chromosome 6

The *Mla* cluster is a complex locus mapped on the top of the chromosome 5 and where 29 alleles have already been reported (Chelkowski et al., 2003; Ramsay et al., 2006) However, this cluster was not found because the new integrated map contained a marker gap in the region of the *Mla* cluster The addition of markers in this region would certainly lead to the finding of correlations related to the presence of *Mla*.

As reported in previous studies, (Chelkowski et al., 2003; Shtaya et al., 2006), no significant associations related to the presence of R genes or QTL for powdery mildew resistance were found on chromosome 6 .

Fig 2.4.g. Association profile of IF and IT the two isolates of Blumeria graminis on chromosome 7. The position of putative QTL is indicated with a black arrow, Mlj with a blue arrow and *Ml(TR)* with a red arrow.




Finally, on chromosome 7, a slight correlation between IF1 and the SSR marker Bmag0223 is observed at 75 cM where the R gene *Mlj* has been reported. The two peaks of IFsusc observed in the same region, between 75 and 85 may be due to the presence of a non

reported QTL clustered with the *Mlj* gene.

Additionally, a series of peaks for the different parameters is observed between 130 and 160 cM. A highly significant correlation can be found between the SSR Bmag0222 and IFsusc allowing to expect the presence of a QTL around 145 cM. It coincided with the position of the reported QTL (Shtaya et al., 2006). 10 to 15 cM away from this QTL, significantly high correlations with IT and IF are found in a region where the *Ml(TR)* gene have been located. The combined presence of Rbgq3 and *Ml(TR)* have been reported before in this region of the chromosome 7 (Shtaya et al., 2006).

### 2.3.5.3. Relations between IF, IT and IT susc

For each marker significantly correlated with powdery mildew reaction, the signs of correlations were compared between every parameter.

Table 2.9. Signs of correlations between mildew parameters

| | | | | |
|---|---|---|---|---|
| IT1 | + | | | |
| IF2 | + | + | | |
| IT2 | + | + | + | |
| IFsusc | - | - | - | - |
| | IF1 | IT1 | IF2 | IT2 |

IF and IT related to one isolate and between isolates were positively correlated. When a QTL or a R gene was represented by a positive association between a mildew parameter and the presence of an AFLP, the other parameters were either positively or not correlated to the presence of this AFLP.

The presence of a gene conferring a type of resistance to a specific mildew isolate (e. g. IF1) did not have a negative pleiotropic effect on the other type of resistance to this isolate (e.g. IT1) or on the reaction to another isolate (ex. IT2 and/or IF2). This gene or QTL is either also efficient to the other isolate (high positive correlation for the parameters) or inefficient (positive correlation for one parameter and no correlation to the other). Concerning partial resistance (IFsusc), a negative correlation with the other parameters associated with the same marker. It is probably due to the fact that part of the QTL for partial resistance to mildew might be allelic variants created by a mutation event occurring in an effective R gene. This theory is supported in several publications (Backes et al., 2003; Shtaya et al., 2006). However, this theory is not valid for all QTL. Many of them are based on completely different mechanisms than the R genes and are, therefore, belonging to two very distinct classes of genes (Qi et al., 1999).

### 2.3.5.4. Prediction of the presence of *mlo* or *MlLa* in cultivars of the collection

The pattern of the marker highly associated with *MlLa* (E42M48-405) was used to

predict the presence of this R gene in cultivars of the collection where they were not reported (Table 2.10). Those data were combined with the reaction of those cultivars to the two isolates of powdery mildew.

Table 2.10. Putative, non-reported presence of *MlLa* in cultivars of the collection deduced from the molecular testing

| Cultivar | R genes already reported | Reaction to I1 and I2 |
|---|---|---|
| Alabama | not reported | Complete resistance |
| Alanis | not reported | Complete resistance |
| Allbright | not reported | Complete resistance |
| Peggy | not reported | Intermediate resistance to I1<br>Complete resistance to I2 |
| Aspen | *mlo* | Complete resistance |
| Bartok | *mlo* | n.d |
| Century | *mlo* | Complete resistance |
| Collie | *Mlg, Mlk, Mla13* | Complete resistance |
| Jacinta | *U* | Complete resistance |
| Madras | *mlo* | Complete resistance |
| Optima | *U* | Complete resistance |
| Orthega | *Mlg, Mla7* | Intermediate resistance to I1<br>Complete resistance to I2 |
| Ricarda | *mlo* | Complete resistance |
| Sabel | *mlo* | Complete resistance |
| Thuringia | *U2, Ml(st)* | Complete resistance |

Two cultivars, ‘Peggy’ and ‘Orthega’ possessed the allele of the AFLP associated with the presence of *MlLa* and showed similar reaction to the two isolates as the cultivars possessing only *MlLa* (Vada). It means that those cultivars may possess either only *MlLa* or *MlLa* combined with R genes that are not effective against I1 and I2. It is probably the case for ‘Orthega’ which possesses already *Mlg*, non effective against I1 or I2 (virulence spectrum) and *Mla7*. *Mla7* does not confer resistance to powdery mildew when it is combined with R genes non effective or conferring incomplete resistance to the isolates. For example, Escort which possesses two non effective R genes (*Mlg* and *Mlk*), *MlLa* and *Mla7* showed susceptibility to I1. Concerning ‘Peggy’, as no information about its R genes was found, it can possess *MlLa* alone or, more likely, a combination of MlLa and other R genes non effective against I1 or I2 (like ‘Orthega’).

For *mlo* and *Mlg*, the same kind of study was not possible. The presence of the allele of interest of a correlated AFLP was not sufficient to draw any conclusion and the type of resistance associated with those genes (the complete resistance for *mlo* and susceptibility for Mlg for both isolates) could be due to the presence of other R genes).

## 2.4. DISCUSSION

### 2.4.1. Map alignment and integration

The new integrated map showed a good general alignment with the previous map. The AFLP and SSR map orders were consistent and the genetic map lengths were quite comparable (appendix 5). This was due to the application of very restrictive selection criteria on the markers. A drawback of this high stringency is that one third of the mapped markers were not conserved in the new map, reducing the amount of information available for the association study. Those losses were partially compensated by the repositioning of unmapped markers. However, a significant risk of placing them at a wrong position existed. The solution to this problem was to place them at their expected position, perform the association study and check for inconsistent results afterwards. As a result, only 15% of the unmapped markers were integrated in the new map (19 out of 113 markers) and 4 of them had to be repositioned on different chromosomes. This retroactive checking had also a negative effect, as the SSR added where chosen to cover only the regions where no common and repositioned markers were present, the discarding of AFLP at the end of the association study led to the appearance of new gaps that remained in the final map. However, despite its lower number of markers, the final integrated map showed a better coverage of the barley genome than the previous map with only 4 gaps of more than 10 cM (Table 2.6). Moreover, the major interest in creating this map was to create a basic map which could be improved later on by using the consensus map of barley as a large source of new markers. Therefore, it was important to keep a maximum number of markers genotyped by Kraakman but it was essential to produce an integrated map which is reliable as it could be used as a basis for further related studies.

### 2.4.2. Confirmation of the results from the previous association study and new results

Generally, the added markers didn't improve the level of significance of correlations because the SSR-traits associations were globally less significant than the AFLP-traits associations. However, the addition of SSR and unmapped AFLPs allowed improving the reliability of several findings of Kraakman et al. (2006) by increasing the number of markers correlated to a specific QTL (see the number of markers associated with the QTLs in Table 2.8a, b and c). Several new putative QTLs more or less reliable (1 or several markers

associated, reported or not in other studies) were also found, bringing new information about the traits studied by Kraakman. in 2006.

#### 2.4.2.1. Leaf rust resistance

When comparing this association study with the one performed by Kraakman for resistance to leaf rust, very similar results were found. All the genes and QTLs derived from association between markers and AUDPC, IT and/or RLP were retrieved in this study including *Rph3* (R gene) and *Rphq2* (QTL). Those QTL were deduced from the same marker-trait correlation found in Kraakman's study (obvious but proof that the statistical analysis was performed correctly) and from associations with markers added in the regions where those QTL were found.

Furthermore, additional reported QTL were found from significant associations between IT (2 QTL) or AUDPC (1 QTL) and SSRs added in the gaps of Kraakman's map. Remarkably, two of those new associations, one involving IT and the other AUDPC were found in the same region of chromosome 6. It indicates that genes related to complete and partial resistance to powdery mildew may be co-located in the same region of chromosome 6. This tight relationship between qualitative (AUDPC) and quantitative (IT) resistance locus has been reported and explained in a previous study (Backes et al., 2003) where possible factors of this phenomenon were explained including the theory of 'defeated R genes'.

#### 2.4.2.2. BYDV

As for the leaf rust resistance, most of the results of Kraakman et al. (2006) were confirmed by this study. Only one QTL was not found back as it corresponds to a gap in the new map. The positioning of some unmapped markers helped to improve the results, allowing the localization of two more QTL. One was linked to a unique marker (could be a false positive) and the other with two markers (more reliable). Two correlations with different SSR markers gave indication of the possible presence of two QTL on chromosome 5 and 6. However, those markers were located in regions were the density was too low to find correlation with neighboring markers that could confirm those observations.

#### 2.4.2.3. DTH and PH

Unlike the other traits, DTH was poorly described in this study whereas numerous QTLs linked to this trait can be found in the literature. Therefore, the study of DTH would require

the use of a map showing a better marker density than this integrated map.

The results found for PH are very comparable to the one found by Kraakman in 2006. Additionally, another QTL was found thanks to the positioning of an unmapped marker correlated with PH.

#### 2.4.2.4. Map integration and consistence of the results

A major limitation of the association mapping method is the lack of consistence between studies. Numerous examples of associations that cannot be replicated can be found in the literature (Cardon et al., 2001). However, in this study it was essential to prove that the results were similar in order to prove that the new created map was reliable and could be used in further studies. Therefore, a major task of this study was to assess if the results from the previous study were conserved after the repositioning of the markers, using the barley consensus map. In deed, very similar results were found. The transfer of markers did not influence much the results from the previous map. As a significant number of AFLP markers were common to the two maps, those AFLPs created a reliable backbone to which new markers could be added. Those new markers were divided in two types, the repositioned AFLPs and the SSR filling the gaps. Concerning the AFLPs, the major risk was to place them at a wrong position. But those risks where reduced to their minimum by applying strict selection criteria. Concerning the SSR-traits correlations, many were found but only the most reliable were described (high significance, reported QTLs). All marker-traits correlations were carefully checked to avoid an over-interpretation of the results, known to be a major source of mistake in association studies (Cardon et al., 2001). In the end, the results found were highly comparable for most of the traits.

### 2.4.3. Value of this association study for describing R genes and QTL for powdery mildew resistance

A major conclusion of the study of Kraakman et al. (2006) was that “LD mapping is a valuable additional tool in the search of applicable markers associations with major genes and QTL” he also added that “LD studies are efficient in indicating novel genes for important agronomic characters” (Kraakman et al., 2006). One aim of this study was to show that the genetic basis of complete and partial resistance to powdery mildew can be described by an association study.

Five R genes (*Mlg*, *MlLa*, Mlj, *Mlf* and *Ml(TR)*) and *mlo*, four reported QTLs and two

new putative QTLs were mapped in this association study. The three parameters linked to the presence of *Mlo*, *MlLa* and *Mlg* in the collection (literature review) allowed locating those three R genes whereas the others R genes were mapped thanks to the associations between IF, IT and markers in regions where R genes had been reported.

Six R genes is a relatively low number when compared to the more than 25 reported. However, association mapping is a young method which needs to be improved and for which several principles of linkage studies cannot be applied. For example, it was not possible to know, beforehand, that the two isolates would show very low virulence to the collection whereas in a linkage study, a cross between a susceptible and a resistant line would, in most of the case, lead to a F2 population segregating for the resistance. However, this association study already gave some interesting results.

The theory of R genes and/or QTL clustering was supported by this study. Several QTL and major genes for powdery mildew resistance were mapped in the same regions. Those clusters can contain several R genes (like in the case of the *Mla* cluster) or R genes and QTLs, like for *Mlf* or *Mlg* (Inukai et al., 2006). They can also include genes for resistance to different pathogen like the co-location of the powdery mildew R genes *MlLa* and leaf rust R gene *Rphg2*.

Three out of the four QTLs found in this study were reported in a study where a test on detached leaves was performed (Backes et al., 2003), The consistency of results between related studies is synonym of a good reliability of the method used.

As a summary, this study gives valuable first step for the analysis of complete and partial resistance to powdery mildew using association mapping. This is a first promising insight but major improvements (see 2.4.5.) would have to be performed in order to complete it.

### 2.4.4. SSR markers and association mapping

SSR markers are useful for association studies because of their practical advantages compared to other types of markers (AFLP, RFLP). They are easy to run, many are available and they required a small quantity of DNA (Karakousis et al., 2003; Ramsay et al., 2000). Moreover, their polyallelic nature makes them more informative compared to the biallelic markers like AFLPs as they offer more possibilities of analyses. Haplotypes studies of SSRs associated with phenotypic data give valuables information on the genetic basis of agronomical traits. They are also more efficient in describing multi-locus structures like

resistance genes clusters. In case of a locus that present many alleles (like the *Ml*a cluster for example), the use of polyallelic markers allows finding possible associations between each of the SSR alleles and each of the locus alleles. This is the reason why SSR are considered as an ideal tool for genetic mapping (Liu et al., 1996) and more particularly for association mapping studies like this one.

However, despite their qualities, it is difficult to perform genetic studies using only SSRs. One reason is that the position of a SSR can be very variable from one study to another, making the integration of SSR-based maps hard to achieve (Karakousis et al., 2003). This study shows that a way to solve this problem is to combine SSRs with other types of markers. Consequently, the map integration becomes easier and variations in the position of SSRs can be critically analyzed. On the other hand, for the study of associations, the statistical treatment of those heterogeneous maps is more complex as biallelic and polyallelic markers must be treated with different statistical models. As a consequence, knowing that two statistical analyses give different outputs in term of significance, it is sometimes necessary to apply different threshold of significance to the outputs. In this study, the SSR-trait correlations appeared to have a lower degree of significance compared to the AFLP-trait correlations.

It is possible to adapt statistical tests in order to apply it to different types of markers and variables (traits) but it requires an advanced level of statistics or, if available, the use of computer programs developed for this purpose.

### 2.4.5. Limitations and improvement for further investigations

Despite the fact that this study gave satisfying results, several limitations decreased the quality of the final results. It is necessary to understand the reasons of those limitations in order to overcome them in further investigations.

First, the presence of gaps reduced the quality of the map. For example, the *Mla* cluster, that is a major cluster of R genes used for resistance to powdery mildew, was not associated with any markers as few were located on the short arm of chromosome 5 (Jorgensen, 1992). Moreover, the estimate of the LD distance, calculated from the significance of correlations between markers, does not take into account phenomena like G x E interactions or gene conversions that are affecting the level of linkage disequilibrium between markers and traits. Moreover, many markers were not in linkage disequilibrium whereas they were located at less than 10 cM from each others (see Fig.2.2a and b). The addition of SSRs and AFLPs, even between markers separated by less than 20 cM, would increase the level of LD between

markers, producing a map of better quality. It would also allow performing multi-locus studies where the level of correlation between a trait and several markers located in the same region can be studied. Those multi-locus studies allow mapping non-reported QTLs with less risks of mistake because a trait correlated with several markers located in the same region is more likely to be linked to the presence of a QTL than when this trait is linked to an isolated marker. Therefore, at the present state, the integrated map is sufficient to confirm the presence of reported genes or QTLs but need to be improved if one desires to map accurately new, non-reported QTLs.

Second, the efficiency of this study was limited by the low aggressiveness of the two powdery mildew isolates. The modern barley cultivars of the collection are the results of breeding programs that include a selection for good level of resistance against the major pathogens of barley, including *Blumeria graminis*. Therefore, a big percentage of the collection possesses one or several major R genes against powdery mildew. A high level of complete resistance is a restrain for the study of partial resistance as the effect of major R genes is masking the effect of QTLs for partial resistance. In order to study partial resistance, the parameter IFsusc was created, but only 10% of the collection were susceptible (IT=4). Therefore, only few significant associations were found for this parameter. To overcome this limitation, further investigation should take into account the R genes present in the collection for the choice of isolate. Several isolates should be chosen in order to have different responses to the major genes. Using isolates that are virulent to the major genes would allow studying more efficiently the genetic basis of partial resistance by having a significantly higher number of plant/pathogen compatible interactions to work on. However, for the *mlo* gene, no virulent isolate has been found so far limiting the study of partial resistance to the remaining 54 % of the collection.

Third, the QTL found in this study are related to partial resistance at the seedling stage but other QTL are involved in other stages of development. To locate those QTLs, data should be collected from infection tests on adult plants for example.

Finally, the R genes study was restrained by the lack of data available for the R gene survey. As a consequence, only the three most represented R genes could be efficiently studied. Completing the survey with information from other sources would probably lead to additional findings.

# 3. GENE ANCESTRY STUDY OF A HITHERTO NON REPORTED LOCUS FOR HIGH TOLERANCE TO BYDV INFECTION IN A COLLECTION OF MODERN TWO-ROW SPRING BARLEY CULTIVARS

## 3.1. INTRODUCTION

### 3.1.1. Barley Yellow Dwarf (BYD)

BYD is a disease caused by a family of virus belonging to the luteovirus group. Those Barley Yellow Dwarf Viruses (BYDV) can affect a broad range of crops (barley, wheat, oat and, to a lower extent, rice and maize) leading to substantial losses of production all over the world. BYDV is transmitted by aphids (vector) into the phloem where it spreads in all parts of the plant. As it needs a vector of transmission, a way of reducing the risks of a BYDV infection is by controlling and monitoring the populations of aphids. But it is a very difficult and expensive method. Therefore, for economical and ecological reasons, the use of tolerant cultivars with satisfactory yields is often preferred (Allen Miller et al., 1997; Friedt et al., 2003).

However, because of the diversity of BYDV pathotypes and its mode of transmission, the traditional methods of breeding for resistance are laborious and expensive. Additionally, no complete resistance to BYDV is known in Barley. Two genes for tolerance to BYDV, ryd1 and ryd2 were found soon after the discovery of the disease. But, due to its low efficiency, ryd1 was rarely used in breeding and only ryd2 was introduced into commercial cultivars (Atlas in 1968 for example (Friedt et al., 2003)). In those conditions, the mapping of genes and QTLs for tolerance to BYDV is of major interest for the breeding companies in order to find new sources of tolerance or resistance and to develop Marker Assisted Selection programs.

### 3.1.2. Gene genealogy study

In gene genealogy, the coalescence theory states that ‘all genes or alleles in a given population are ultimately inherited from a single ancestor called the Most Recent Common Ancestor shared by all the carriers of that particular allele in the population’ (source:

Wikipedia). This principle was used to reconstruct the ancestral relationship and find the original donor of a recently reported gene conferring high tolerance to BYDV in 15 cultivars of a modern, two-row spring barley collection (Kraakman et al., 2006). This locus has been found to be strongly associated with the allele 158 of the SSR marker HVM054 (Kraakman et al., 2006). This marker was used to test the candidates selected from the ancestry study and confirm if they were the donor of the gene or not.

The method using the coalescence theory to reconstruct the genealogy of genes for resistance to pathogen is gaining an increasing interest in the research community (Schaal et al., 2000). This has led to the development of algorithms to study complex cases. However, those new tools were not used in this study as the set of studied cultivar is relatively small and the genealogy was focus on a single locus.

## 3.2. MATERIAL AND METHODS

### 3.2.1. Phylogenetic study

The phylogenetic study was performed by collecting information about the ancestors of the 15 cultivars carrying the gene in the collection (Kraakman et al., 2006). Information was collected up to 8 generations in several on line databases (see reference “Databases)”and with the help of the software PEDITREE. The efforts were focused on the branches that were leading to common ancestors of 2 or more of the 15 cultivars. Branches that were not leading to common ancestors were discarded. In the end, a selection of ancestors and intermediate ancestors was created and those candidates were tested with a molecular marker.

### 3.2.2. The molecular testing

#### 3.2.2.1. DNA material

The seeds from of ancestry candidates were found in the barley collection of the department or ordered to gene banks. Four seeds were planted for each cultivar in 14cm diameter pots. After 14 days, the DNA was extracted following the protocol described in 2.2.6.1.

#### 3.2.2.2. The marker HVM054

The marker used for the molecular testing was the SSR showing high association with

BYD in Kraakman's study: HVM054 (Kraakman et al., 2006). It is a polyallelic marker located on chromosome 2(2H) which showed 5 different alleles in a size range from 150 to 162 base pairs (bp) (Table 3.1).

#### 3.2.2.3. PCR mix and condition

The PCR mix and conditions are the same as described in 2.2.6.3 and, as indicated in table 3.1, the PCR profile A was used (Appendix 3).

Table 3.1. Description of the SSR marker HVM054

| | |
|---|---|
| Name | **HVM054** |
| PBR-Nb[1] | **12** |
| PCR[2] | **A** |
| Label (IRD) | **800** |
| Quality[3] | **5** |
| Size range (bp) | **149-165** |
| Chromosome | **2 (2H)** |
| Position (cM)[4] | **122** |
| PIC | **1** |
| Forward primer | **AACCCAGTAACACCTGTC** |
| Unlabeled | **CTG** |
| Reverse primer IRD-labeled | **AGTTCCCTGACCCGATGT C** |
| Reference | **(Liu Z.W., 1996)** |

[1] Code used in the lab
[2] PCR profile used for barley SSR, (see appendix 3)
[3] Quality rating: (observed in the L94xVada population and according to the scale of (Smulders et al., 1997), 5: very weak bands or no amplification at all.
[4] Approximate

#### 3.2.2.4. Gel running

The gel was run and analyzed following the same protocol as explained in 2.2.6.5.

## 3.3. RESULTS

### 3.3.1. The 15 BYDV tolerant cultivars

Information about the origin, the year of introduction and the parental lines of the 15 cultivars were collected from databases and literature (Table 3.2.).

The 15 cultivars were released between 1983 (Adele) and 2001 (Cicero). They were mostly varieties developed in the United Kingdom. 10 cultivars out of the 15 had been developed by the company *New Farm Crop Ltd.* located in Lincolnshire, England. It seems like this specific gene for tolerance to BYDV was part of the breeding program of this

company, as a consequence, it is possible that the common ancestor is a cultivars that was released by this company or a parental line used to create the most recent varieties.

Table 3.2. Description of the 15 cultivars of interest.

| Cultivar | Year of release | Breeding company[1] | Parents |
|---|---|---|---|
| ABELONE | n.d. | NFC | Corn x Force x Troop |
| ADELE | 1983 | PBI | Aramir x Perfekta x Annemarie |
| AKTA | 1984 | Abed | Loti x *Abed 1657* [4] |
| ASPEN | 1999 | Nickerson | Vintage[2] x Chariot |
| BREWSTER | 1994 | NFC | Troop x *Br 3459* |
| BRISE | 2000 | NFC | **Optic**[3] x **Chalice** x *NFC 94-20* |
| CHALICE | 1997 | NFC | (**Cooper** x *NFC 514*-5) x Chariot |
| CICERO | 2001 | Sejet | **Chalice** x Prolog |
| CORK | 1993 | NFC | ((Dera x Fleet) x Trumpf) x Peel |
| COOPER | 1994 | NFC | Corniche x force x Troop |
| EXTRACT | 1996 | NFC | Cask x Chariot x Amber |
| LUX | 1999 | Sejet | **Cork** x Goldie |
| OPTIC | 1993 | NFC | Chad x Corniche x Force |
| TEAL | 1993 | NFC | Natasha x *NFC 461*81 x Fleet |
| STATIC | 1998 | NFC | Landlord x **Cork** |

[1] NFC: New Farm Crop Ltd. (UK), PBI: Plant Breeding International Ltd. (UK), Abed: Abed Plant Breeding (DK), Nickerson UK Ltd. (UK), Sejet: Sejet Planteforædling (DK)

[2] in red, the cultivars present in the collection and that are known not to possess the allele of interest of the marker (Kraakman et al., 2006).

[3] in bold, cultivars that are known to possess the allele of interest.

[4] in italic, cultivars that could not be described as their name is a code specific to the plant breeder.

One of the 15 cultivars was Aspen. But, from the molecular test it had not been possible for Kraakman to decide whether this cultivar was carrying the allele 158, 167 or both (kraakman et al., 2006). In this genealogy study, as mentioned in the table 3.2, it was found that Aspen was the result of a cross between two cultivars belonging to the collection which were not carrying allele of interest. Therefore, it was decided not to included Aspen in the further steps of the ancestry study as it most probably does not have the gene of interest. Five of the most recent cultivars had one or two of the other 11 cultivar as parent (Brise, Chalice, Cicero, Lux, and Static). Therefore, they were not informative for the ancestry study. In the end, the gene genealogy focused on 9 informative cultivars. A cultivar named ‘Akita’ was also part of the set. However, this name was not found in any database. Instead, a cultivar called Akta was found. As it is a modern (1984) two-row barley cultivar released in Western Europe, it was considered that ‘Akta’ and ‘Akita’ are actually the same cultivar.

### 3.3.2. Candidate ancestors selected from literature and databases

Candidates were selected in the ancestry of the 15 cultivars up to five generations using literature, databases and the PEDITREE software. The list of the cultivars tested with the year of release is presented in table 3.3a. Gull and Binder, were the oldest candidates and were part of the ancestry of the entire set. However, the molecular testing revealed that none of them

carried the allele HVM54-158.

Table 3.3a. Description of the 'old' ancestry candidates tested

| Candidate | Year of release | Breeder Institution | Country |
|---|---|---|---|
| BINDER | 1916 | Abed Plant Breeding | Denmark |
| GULL | 1929 | Svalof Weibull | Sweden |
| KENYA | 1931 | Abed Plant Breeding | Denmark |
| EMIR | 1962 | Cebeco Handelsraad | The Netherlands |
| PROCTOR | 1952 | Cambridge Laboratory IPSR[1] | England |
| DELTA | 1959 | Cebeco Handelsraad | The Netherlands |

[1] Institute of Plant Sciences Research

At this point, the strategy changed: Instead of trying to find directly the most recent common ancestor of the whole set, the search was focused on so called 'intermediate' ancestors that where found in the ancestry of at least 2 cultivars. Those candidates were parents or grandparents of the cultivars. This technique can be defined as a step by step search were wrong branches can be discarded early, increasing the chance of finding the donor of the allele by focusing on the branches were the allele is found. By chance, in a scientific paper on the development of the spring barley germplasm, one of the candidates (Fleet) and two of his ancestors (Midas and Goldmarker) had been tested for the marker HVM54 and the results seemed to indicate that those cultivar possessed HVM54-158 (Russell et al., 2000).

Table 3.3b. Description of the 'intermediate ancestry candidates' tested

| Ancestry candidates | Year | Breeder institution | Progeny |
|---|---|---|---|
| FLEET | 1985 | New Farm Crop Ltd. (UK) | **TEAL**[1] , **CORK**[2], LUX, STATIC, OPTIC, BRISE, EXTRACT |
| GOLDMARKER | 1977 | The Miln Masters Group, Ltd. (UK) | **Fleet** |
| MIDAS | 1970 | The Miln Masters Group, Ltd. (UK) | **Goldmarker** |
| TROOP | nd | New Farm Crop Ltd. (UK) | **COOPER**, **BREWSTER**, **ABELONE**, CHALICE, CICERO, BRISE |
| CLARET | 1978 | Nickerson RPB Ltd. (UK) | **Fleet**, **Regatta**, **Troop** |
| REGATTA | 1985 | Cebeco Handelsraad (NL) | CORK, LUX, STATIC, EXTRACT |
| NATASHA | 1984 | Unisigma (FR) | **TEAL**, EXTRACT |
| CORNICHE | 1988 | Volkseigenes Gut-Saatzucht Bernburg (GER) | **OPTIC**, BRISE, EXTRACT |
| KASKADE | 1981 | Verwaltungsstelle Weihenstephan (GER) | **Troop** |
| ARAMIR | 1972 | Unisigma (FR) | **ADELE**, Natasha |
| LOFA ABED | 1970 | Abed Plant Breeding (DK) | AKTA |
| MINERVA | 1955 | Foundation for Agricultural Plant Breeding (NL) | **Lofa** |

[1] In Bold, direct progeny: cultivars for which the ancestry candidate is a parent of first generation
[2] In capital, cultivars of interest

A total of 12 candidates were tested. Fleet, Troop, Natasha and Aramir are cultivars that are direct parents of one or several of the 15 cultivars. The others are ancestors of 2$^{nd}$ or 3$^{rd}$ generation. For Akta, no common ancestor with other cultivars were found but two cultivars in its ancestry were tested to give indications on which branch should be studied more in details.

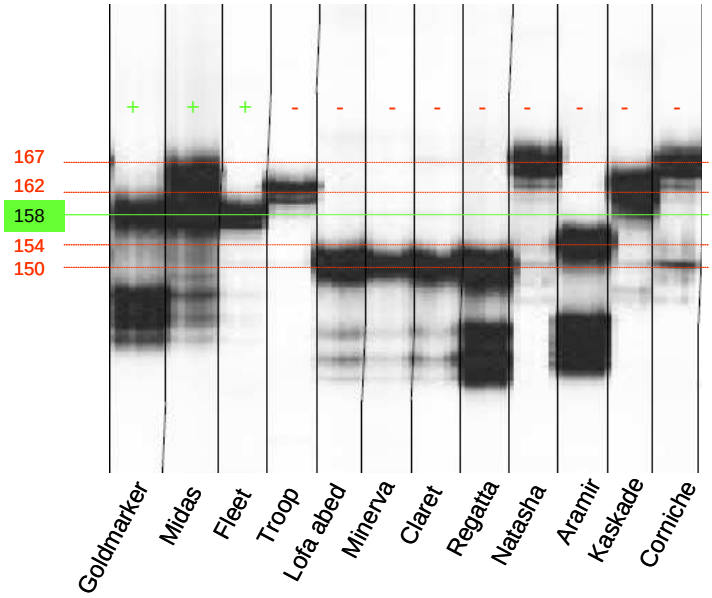



Fig.3.1. HVM54 fingerprint for the selected intermediate ancestry candidates. Goldmarker, Midas and Fleet are the only tested cultivars showing the allele of interest HVM54-158.

Out of the 12 cultivars tested, only Midas, Goldmarker and Fleet carried HVM54-158 (Fig 3.1), confirming that the allele of HVM54 found for those cultivars (and some others) in the literature was, in deed, the allele of interest (Russell et al., 2000). Fig.3.2 is a progeny tree gathering both positive and negative results form the molecular test.

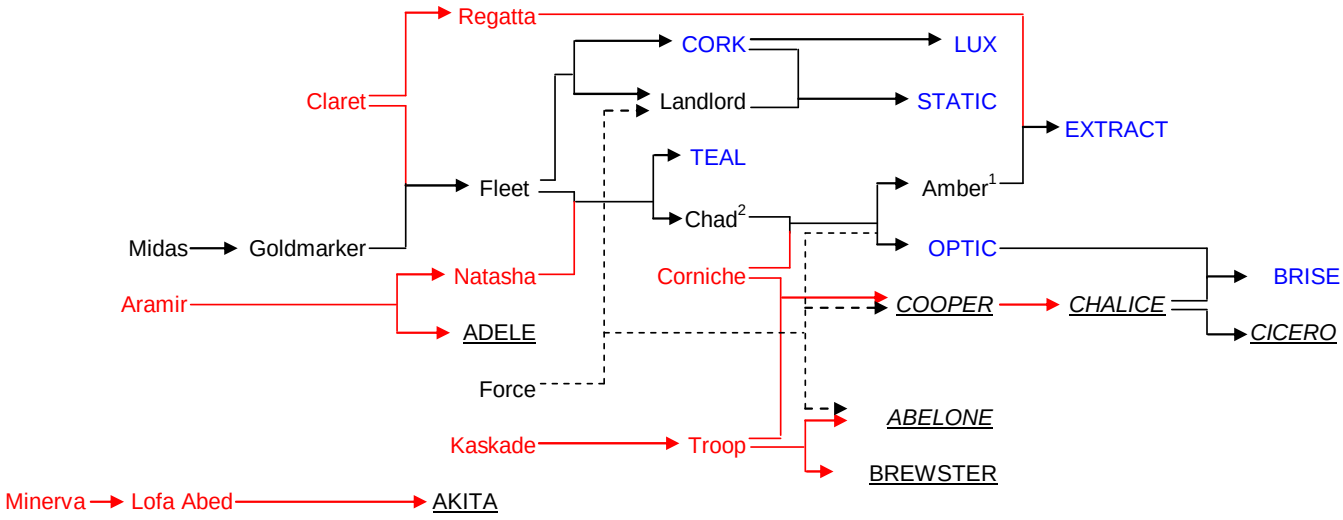



Fig. 3.2. Ancestry tree of the 14 cultivars constructed from the tested candidates.
This phylogenetic tree is based on the literature search for candidates and the molecular test for HVM54.
In capital letters, the 14 cultivars showing high tolerance to BYDV.
In blue, the one cultivars for which the most recent common ancestor was found.
In red, cultivars that did not carry the specific allele of the marker HVM54
Underlined, the cultivars of interest for which the source of the gene was not confirmed
In green, Force and the cultivars of interest which are part of its progeny.
In dash lines the branch derived from 'Force'. In italic, the cultivars which would be explained if Force is proven to carry HVM54-158

[1] Cultivar that have not been tested but which is known to have a high level of tolerance to BYDV (Banwo et al., 1998).

[2] Cultivars that possess the same allele of HVM54 as Midas, Goldmarker, Fleet, Optic and Cooper (Russell et al., 2000).

The three confirmed cultivars were all part of the same phylogenetic branch, giving the origin of the gene for 7 cultivars out of the 14. Midas was the oldest ancestor found. This cultivar was released in 1970 in the United Kingdom and was obtained by multiple crosses between 4 parents: a radio mutant of Proctor (UK), Wong (CHN), a mutant of Maythorpe (UK) and cultivar called Mildew Resistant A. The MRCA of the 7 cultivars was Fleet. It was separated by a maximum of 3 generations from the cultivars studied (Extract, Brise).

For the cultivars not included in this branch, Adele and Akta were old cultivars that were released at the same time Fleet. Midas and Goldmarker were not among their ancestors. Therefore, the link between those cultivars and the main branch would be found between ancestors of Midas and one of the ancestors of Adele and Akta.

As Corniche and Troop were not carrying the allele, the donor of the gene for Abelone, Brewster, Cooper, Chalice and Cicero was not found. But, a cultivar named 'Force', not tested in this study, could be the source of the allele for them. In fact, Force was one of the three cultivars crossed to obtain Cooper. It was crossed with Corniche and Troop which both were not the source of the allele. Additionally, Force was found in the ancestry of Abelone, Optic, Amber and Landlord which were all proved or reported to carry HVM54-158 (fig.3.1). Furthermore, Force was released in 1981 by *New Farm Crop Ltd.*, the company that released most of the 14 cultivars carrying the gene of interest (Table 3.2). Despite those arguments, as no link with the 'Midas branch' was found in the ancestry of Force, a molecular test would be necessary to confirm if it does carry HVM54-158. This would confirm it as the donor of the gene for this group of cultivars. In case it is verified, the link between the two branches could be further investigated. It was not possible to obtain this cultivar from germplasm collections

Finally, concerning Brewster, as Troop was found not to be the source of the allele, the allele was inherited from its other parent 'Br 3459'. However, cultivars with this type of accession codes, given by companies are difficult to study as very little information is available about them.

## 3.4. DISCUSSION

This analysis of the ancestry resulted in the finding of common ancestor of 7 cultivars. Despite the fact that this study failed in finding the complete ancestry of the gene, it represents a valuable step further in this direction. In fact, the phylogenetic branch originating from Midas explains 50 % of the genealogy of the locus of interest (7 cultivars out of 14) which is already a satisfying result. Moreover, there are strong indications that Force would carry the allele of interest. If it is confirmed, its corresponding phylogenetic branch would

explain the origin of the gene for 4 additional cultivars.

The Most Recent Common Ancestor of this set of cultivar is older than the oldest ancestor studied, meaning that it is a cultivars that was bred in the 60s or earlier. The search for such old ancestry is made difficult by the lack of data and the limited availability of seeds for testing. It is a study that requires time and help from germplasm banks and breeding companies (*New Farm Crop Ltd.*).

Surprisingly, this major gene for tolerance to BYDV has not been spread widely into the Western Europe germplasm (only 15 cultivars out of 148) or reported in other studies despite its valuable effect on resistance for this important. As shown in the present study, this gene has been present in British cultivars for more than 30 years before being found back in Danish varieties (Lux in 1999 and Cicero in 2001 both released by Sejet Planteforædling). This gene was probably introduced for the first time in British cultivars, more than 40 years ago, and remained in the British germplasm for a long time before being introduced recently in cultivars from other countries.

## 3.5. SUGGESTION FOR FURTHER STUDIES

The first task of a further study should be to confirm that Force is a donor of the gene. When it is confirmed, the effort should be focused on finding the common ancestor of the two phylogenetic branches of Midas and Force and try to connect Adele and Akta to ancestors in this phylogenetic tree. When the complete tree is constructed, the evolution of the gene over generations could be studied. Valuable pieces of information could probably be obtained from the breeding company *New Farm Crop Ltd.* as this gene for tolerance to BYDV was conserved in their commercial varieties from Fleet in 1985 to Brise in 2000. It is a breeding company funded in 1979 in Lincolnshire (England), which now belongs to Syngenta.

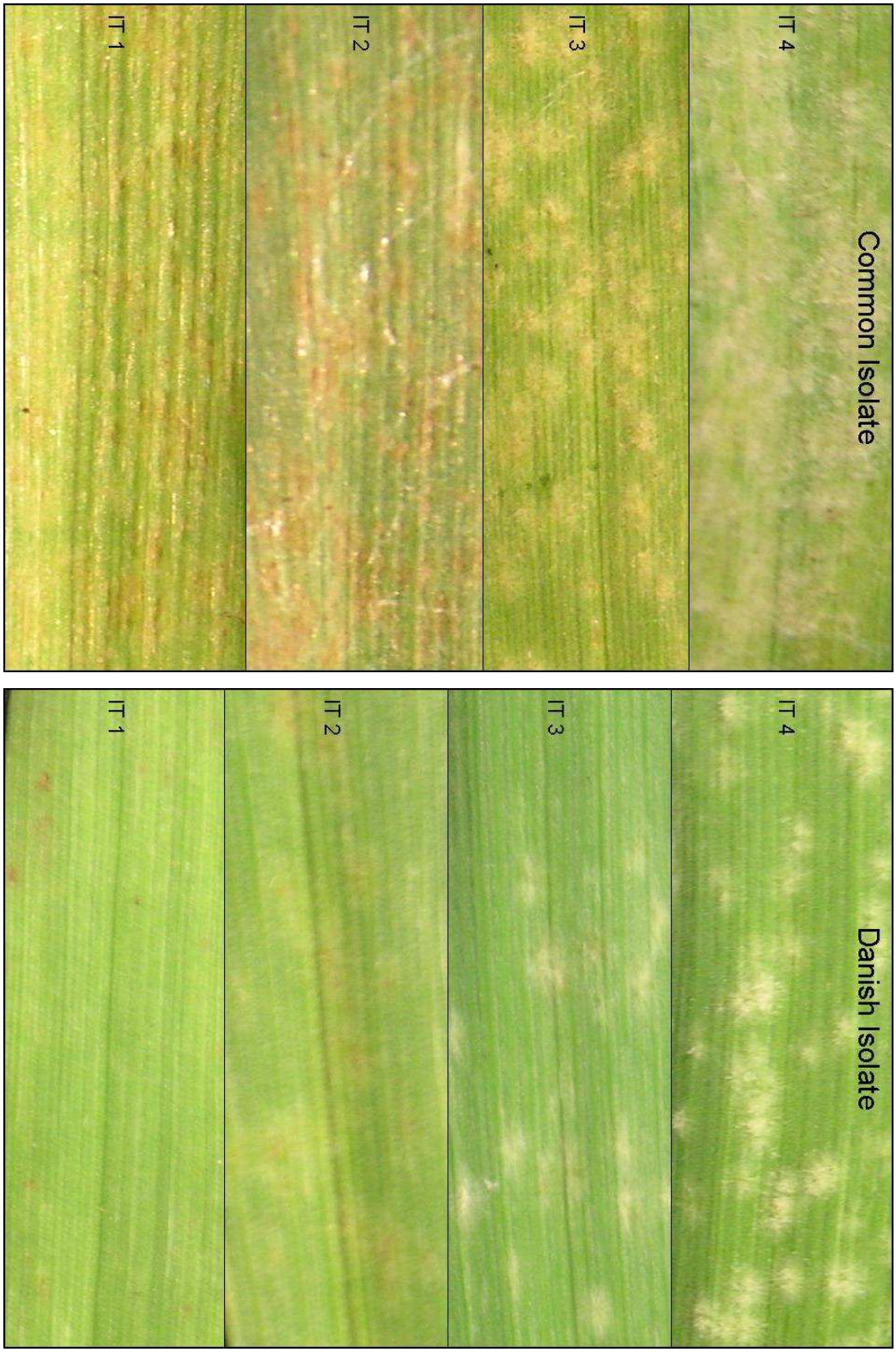
# 4. APPENDIXES

## APPENDIX 1: Tables and figures

### Tables

# Figures

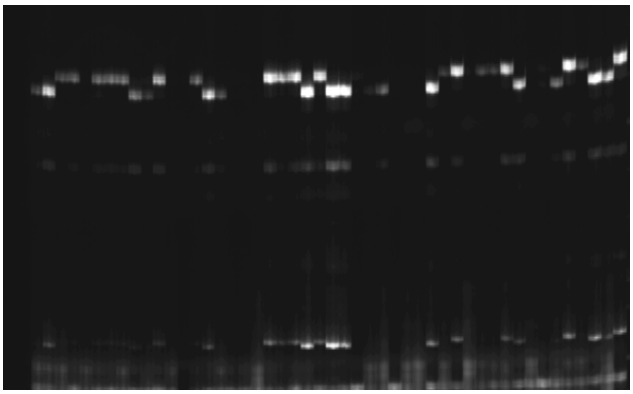
APPENDIX 2: Infection types observed for the two isolates.



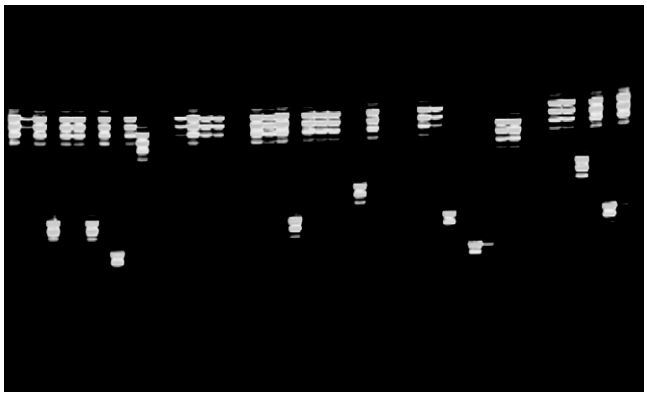
# APPENDIX 3: PCR Profiles

| | Number of cycles | Duration | Temperature (in $^{o}$C) |
|---|---|---|---|
| **Profile A** | | | |
| | 1 cycle | 3 minutes | 94 |
| | 10 cycles | 30 seconds | 94 |
| | | 30 seconds | **60 to 55, decrease of 0.5$^{o}$C each cycle** |
| | | 15 seconds | 72 |
| | 35 cycles | 30 seconds | 94 |
| | | 30 seconds | **55** |
| | | 15 seconds | 72 |
| | 1 cycle | 5 minutes | 72 |
| **Profile B** | | | |
| | 1 cycle | 3 minutes | 94 |
| | 10 cycles | 30 seconds | 94 |
| | | 30 seconds | **63 to 58, decrease of 0.5$^{o}$C each cycle** |
| | | 15 seconds | 72 |
| | 35 cycles | 30 seconds | 94 |
| | | 30 seconds | **58** |
| | | 15 seconds | 72 |
| | 1 cycle | 5 minutes | 72 |
| **Profile C** | | | |
| | 1 cycle | 3 minutes | 94 |
| | 10 cycles | 30 seconds | 94 |
| | | 30 seconds | **65 to 60, decrease of 0.5$^{o}$C each cycle** |
| | | 15 seconds | 72 |
| | 35 cycles | 30 seconds | 94 |
| | | 30 seconds | **60** |
| | | 15 seconds | 72 |
| | 1 cycle | 5 minutes | 72 |

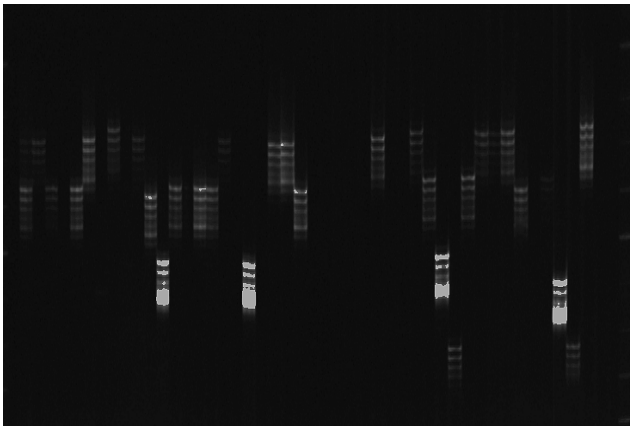
# APPENDIX 4: Examples of SSR fingerprints obtained



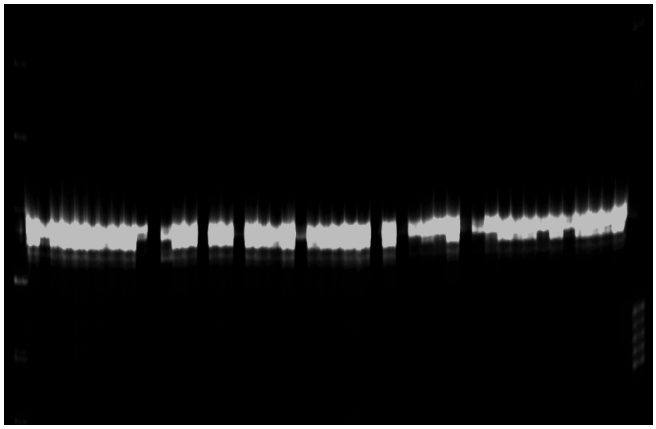
Bi-allelic SSR marker



Poly-allelic SSR marker



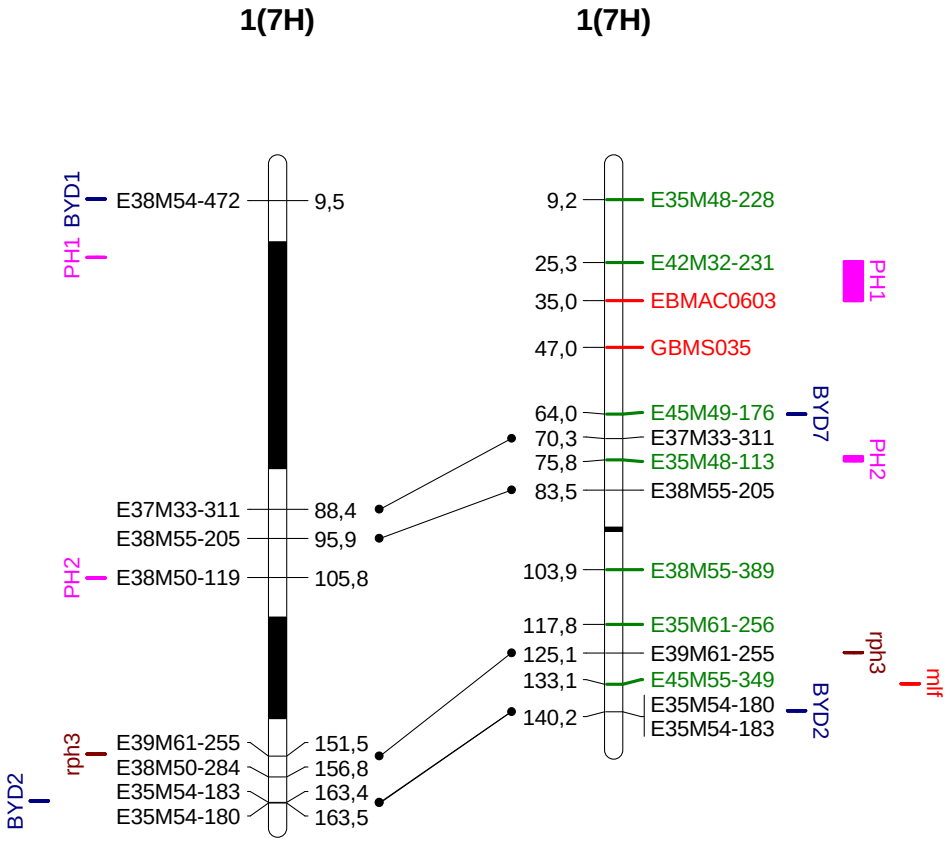
Poly-allelic, highly polymorphic SSR marker

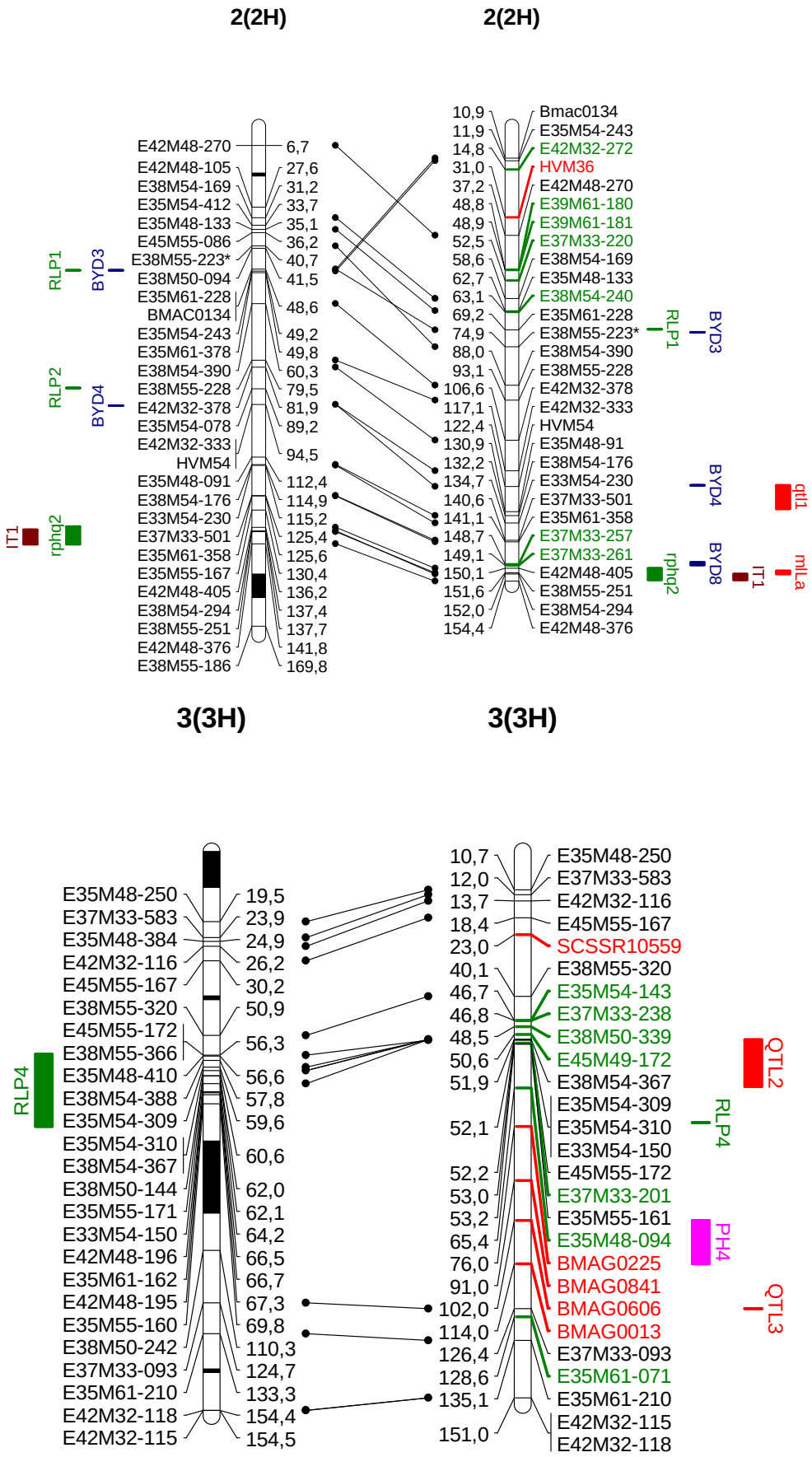


Non-polymorphic SSR marker

## APPENDIX 5: Maps comparison.

For each of the chromosomes, the previous integrated map is presented on the left side and contains markers position and the QTL found by Kraakman et al. (2006). On the right side, the new integrated map with the position of the common markers (in black), the repositioned markers from Kraakman's study (in green) and the added SSR markers (in red). Common markers between the two maps are connected by a line to show the alignment. QTL and genes are also represented, for powdery mildew, R genes are given, reported QTL are shown as "QTL" and non-reported QTL as "qtl".

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2(2H)
E42M48-270 6,7
E42M48-105 27,6
E38M54-169 31,2
E35M54-412 33,7
E35M48-133 35,1
E45M55-086 36,2
E38M55-223* 40,7
E38M50-094 41,5
E35M61-228 48,6
BMAC0134
E35M54-243 49,2
E35M61-378 49,8
E38M54-390 60,3
E38M55-228 79,5
E42M32-378 81,9
E35M54-078 89,2
E42M32-333 94,5
HVM54
E35M48-091 112,4
E38M54-176 114,9
E33M54-230 115,2
E37M33-501 125,4
E35M61-358 125,6
E35M55-167 130,4
E42M48-405 136,2
E38M54-294 137,4
E38M55-251 137,7
E42M48-376 141,8
E38M55-186 169,8
RLP1
BYD3
RLP2
BYD4
IT1
rphq2
10,9 Bmac0134
11,9 E35M54-243
14,8 E42M32-272
31,0 HVM36
37,2 E42M48-270
48,8 E39M61-180
48,9 E39M61-181
52,5 E37M33-220
58,6 E38M54-169
62,7 E35M48-133
63,1 E38M54-240
69,2 E35M61-228
74,9 E38M55-223*
88,0 E38M54-390
93,1 E38M55-228
106,6 E42M32-378
117,1 E42M32-333
122,4 HVM54
130,9 E35M48-91
132,2 E38M54-176
134,7 E33M54-230
140,6 E37M33-501
141,1 E35M61-358
148,7 E37M33-257
149,1 E37M33-261
150,1 E42M48-405
151,6 E38M55-251
152,0 E38M54-294
154,4 E42M48-376
RLP1
BYD3
BYD4
BYD8
rphq2
IT1
qtl1
mlLa
3(3H)
3(3H)
E35M48-250 19,5
E37M33-583 23,9
E35M48-384 24,9
E42M32-116 26,2
E45M55-167 30,2
E38M55-320 50,9
E45M55-172 56,3
E38M55-366
E35M48-410 56,6
E38M54-388 57,8
E35M54-309 59,6
E35M54-310 60,6
E38M54-367
E38M50-144 62,0
E35M55-171 62,1
E33M54-150 64,2
E42M48-196 66,5
E35M61-162 66,7
E42M48-195 67,3
E35M55-160 69,8
E38M50-242 110,3
E37M33-093 124,7
E35M61-210 133,3
E42M32-118 154,4
E42M32-115 154,5
RLP4
10,7 E35M48-250
12,0 E37M33-583
13,7 E42M32-116
18,4 E45M55-167
23,0 SCSSR10559
40,1 E38M55-320
46,7 E35M54-143
46,8 E37M33-238
48,5 E38M50-339
50,6 E45M49-172
51,9 E38M54-367
52,1 E35M54-309
E35M54-310
E33M54-150
52,2 E45M55-172
53,0 E37M33-201
53,2 E35M55-161
65,4 E35M48-094
76,0 BMAG0225
91,0 BMAG0841
102,0 BMAG0606
114,0 BMAG0013
126,4 E37M33-093
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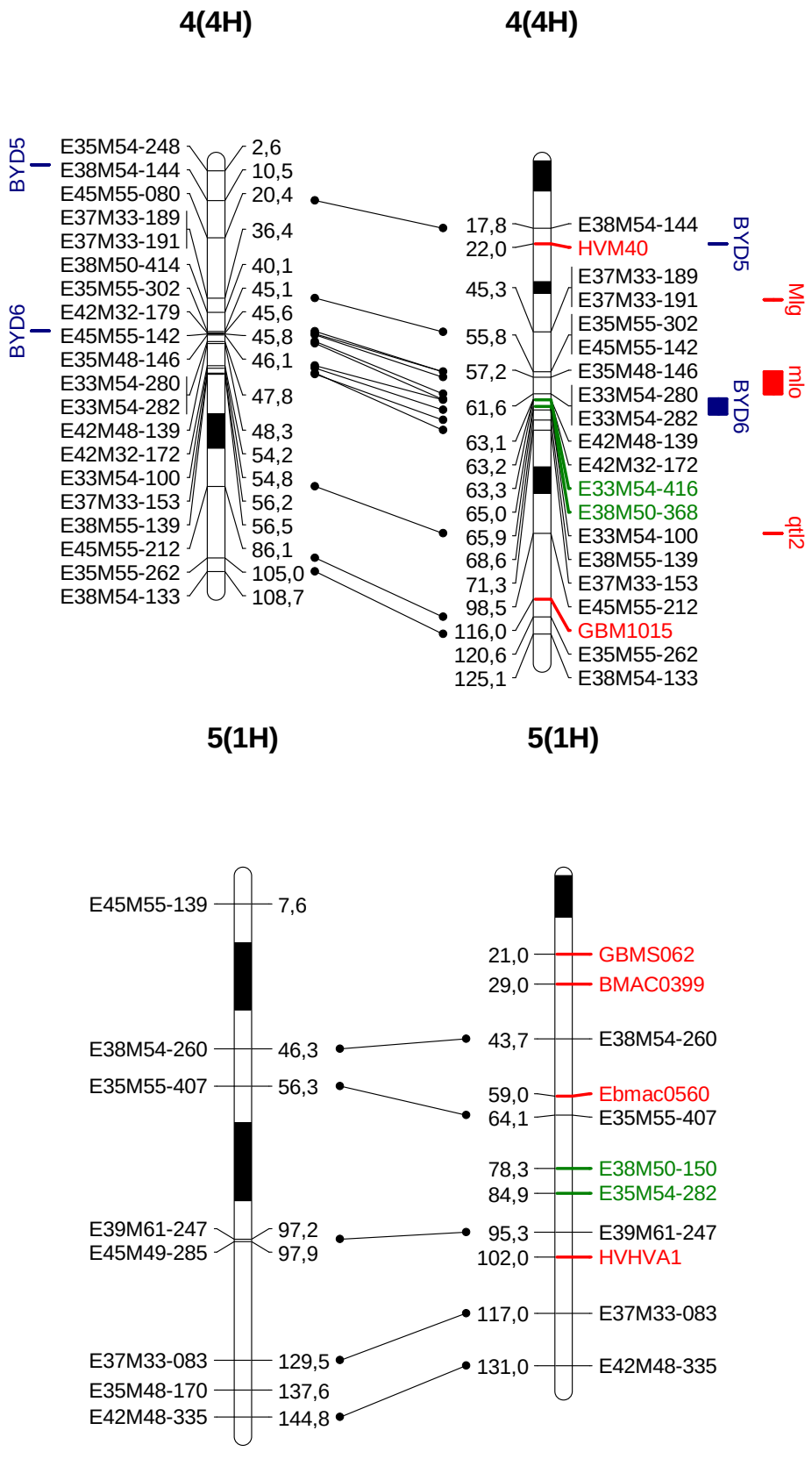
135,1 E35M61-210
151,0 E42M32-115
E42M32-118
QTL2
RLP4
PH4
QTL3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BYD6
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E38M50-414
E35M55-302
E42M32-179
E45M55-142
E35M48-146
E33M54-280
E33M54-282
E42M48-139
E42M32-172
E33M54-100
E37M33-153
E38M55-139
E45M55-212
E35M55-262
E38M54-133
2,6
10,5
20,4
36,4
40,1
45,1
45,6
45,8
46,1
47,8
48,3
54,2
54,8
56,2
56,5
86,1
105,0
108,7
17,8
22,0
45,3
55,8
57,2
61,6
63,1
63,2
63,3
65,0
65,9
68,6
71,3
98,5
116,0
120,6
125,1
E38M54-144
HVM40
E37M33-189
E37M33-191
E35M55-302
E45M55-142
E35M48-146
E33M54-280
E33M54-282
E42M48-139
E42M32-172
E33M54-416
E38M50-368
E33M54-100
E38M55-139
E37M33-153
E45M55-212
GBM1015
E35M55-262
E38M54-133
BYD5
BYD6
Mlg
mlo
qtl2
5(1H)
5(1H)
E45M55-139
E38M54-260
E35M55-407
E39M61-247
E45M49-285
E37M33-083
E35M48-170
E42M48-335
7,6
46,3
56,3
97,2
97,9
129,5
137,6
144,8
21,0
29,0
43,7
59,0
64,1
78,3
84,9
95,3
102,0
117,0
131,0
GBMS062
BMAC0399
E38M54-260
Ebmac0560
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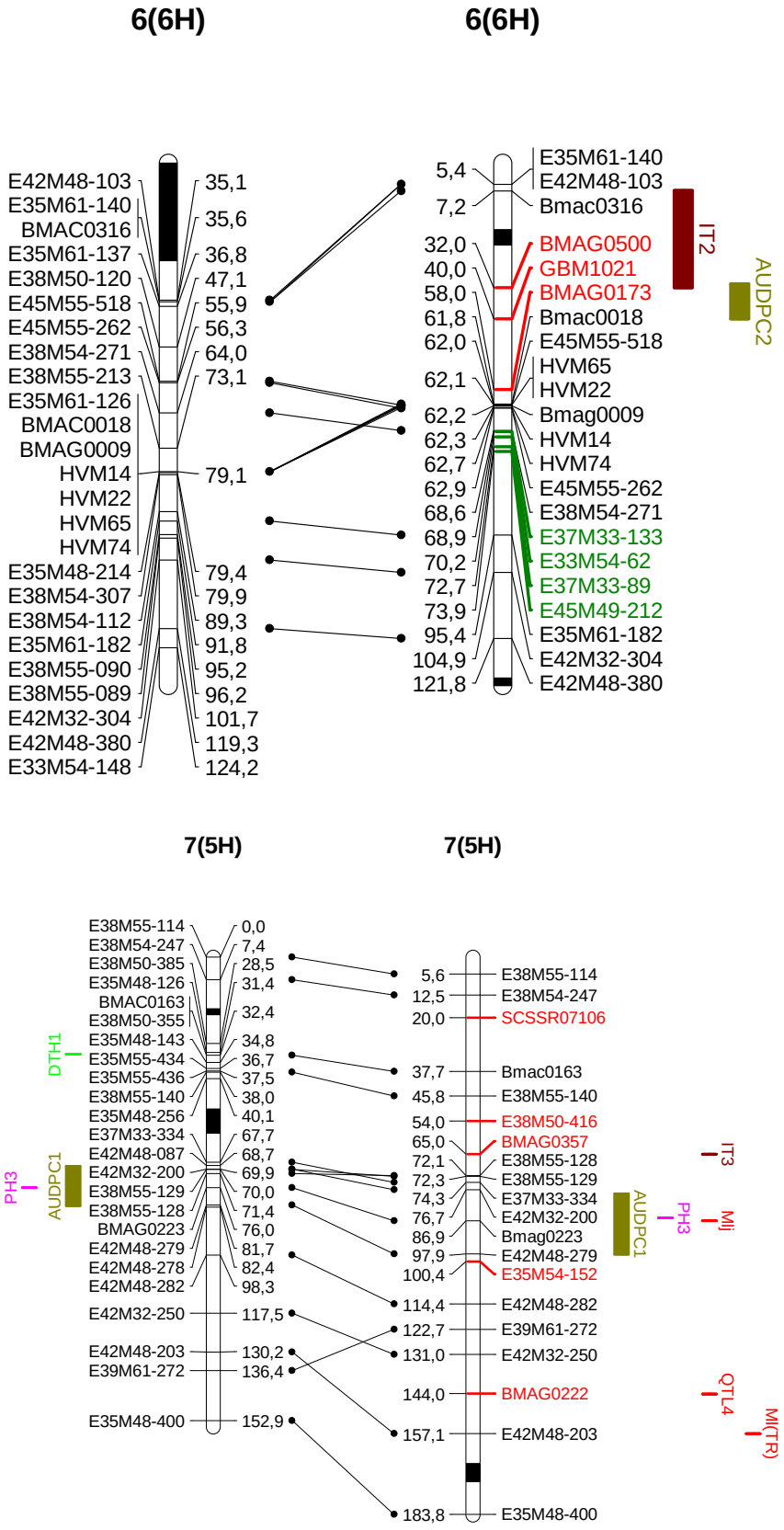
E38M50-150
E35M54-282
E39M61-247
HVHVA1
E37M33-083
E42M48-335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6(6H)
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E38M50-120
E45M55-518
E45M55-262
E38M54-271
E38M55-213
E35M61-126
BMAC0018
BMAG0009
HVM14
HVM22
HVM65
HVM74
E35M48-214
E38M54-307
E38M54-112
E35M61-182
E38M55-090
E38M55-089
E42M32-304
E42M48-380
E33M54-148
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35,6
36,8
47,1
55,9
56,3
64,0
73,1
79,1
79,4
79,9
89,3
91,8
95,2
96,2
101,7
119,3
124,2
5,4
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40,0
58,0
61,8
62,0
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62,2
62,3
62,7
62,9
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68,9
70,2
72,7
73,9
95,4
104,9
121,8
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Bmac0316
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GBM1021
BMAG0173
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HVM65
HVM22
Bmag0009
HVM14
HVM74
E45M55-262
E38M54-271
E37M33-133
E33M54-62
E37M33-89
E45M49-212
E35M61-182
E42M32-304
E42M48-380
IT2
AUDPC2
7(5H)
7(5H)
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E38M54-247
E38M50-385
E35M48-126
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E38M50-355
E35M48-143
E35M55-434
E35M55-436
E38M55-140
E35M48-256
E37M33-334
E42M48-087
E42M32-200
E38M55-129
E38M55-128
BMAG0223
E42M48-279
E42M48-278
E42M48-282
E42M32-250
E42M48-203
E39M61-272
E35M48-400
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28,5
31,4
32,4
34,8
36,7
37,5
38,0
40,1
67,7
68,7
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76,0
81,7
82,4
98,3
117,5
130,2
136,4
152,9
DTH1
PH3
AUDPC1
5,6
12,5
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37,7
45,8
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65,0
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72,3
74,3
76,7
86,9
97,9
100,4
114,4
122,7
131,0
144,0
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183,8
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E38M54-247
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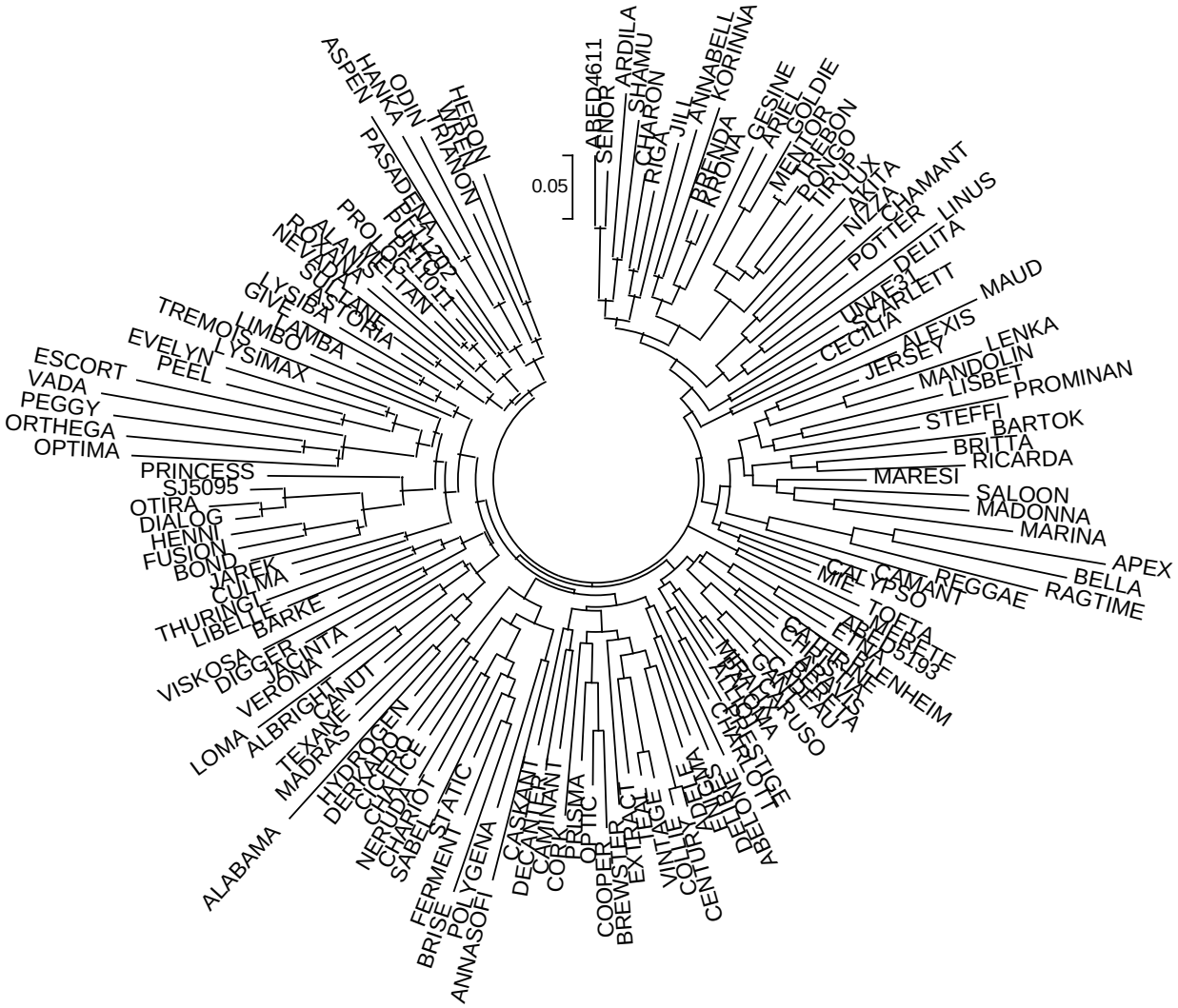
Bmac0163
E38M55-140
E38M50-416
BMAG0357
E38M55-128
E38M55-129
E37M33-334
E42M32-200
Bmag0223
E42M48-279
E35M54-152
E42M48-282
E39M61-272
E42M32-250
BMAG0222
E42M48-203
E35M48-400
IT3
PH3
Mj
AUDPC1
QTL4
MI(TR)

APPENDIX 6: Neighbor Joining (NJ) circular relation tree for the collection



APPENDIX 7: Command file used for the correlation between SSR and the continuous traits.

```
text xlsfile;valu='Mathieu.xls'
text tsheet;valu='genstat'
scal npheno;valu=6
text seperator;'M'   "Use the M between the numbered ssr marker and the size of the allele as a separator"

\**************************************************************************************
\** Read in the data
impo [prin=*] xlsfile;sheet=tsheet;isav=p
calc np,len=nval(p,p[1])
prin !(1...np),p;just=r,l;deci=0

calc start=npheno+1
vari [valu=start...np] vssr

prin [chan=tssr;ipri=*;squa=yes] !p(p[#vssr]);just=l;skip=0;fiel=1
prin [chan=phenoname;ipri=*;squa=yes] !p(p[1...npheno]);just=l;skip=0;fiel=1
prin phenoname
prin tssr;just=l

calc vmark=getp(tssr;seperator)
conc [mark] tssr;width=vmark-1
conc [allel] tssr;skip=vmark-1;widt=-1

prin tssr,mark,allel;just=l;fiel=20

grou mark;fact=fmark;labe=labmark
prin labmark
FSUB fmark;subf=fallel
calc nmark,maxallel=nlev(fmark,fallel)
prin nmark

tabu [clas=fmark,fallel;coun=tel] vssr;tota=tvssr
prin tel;deci=0;fiel=8
tabu [clas=fmark;count=telallel]
prin telallel
vari vtelallel;valu=telallel;deci=0

\** save the columns in the spreadsheet where each ssr marker was from
prin tvssr;deci=0;fiel=8
\** Separate variates for each marker, containing the nrs. of Excel columns where the allele scores can be found
vari [nval=maxallel] ppos[1...nmark]
equa tvssr;ppos
```

```
prin ppos[1...nmark];deci=0;fiel=6

\** put ssr data in pointers, with size of the number of alleles
for i=1...nmark
 subs [cond=ppos[i].ne.cons('*')] ppos[i];suballel[i]
 poin [valu=p[#suballel[i]]]] pssr[i]
 \prin pssr[i]
endf
vari [modi=yes] p[#vssr];deci=0

\** example
prin p[1],pssr[1,nmark][];fiel=15

scalar adjr2, fprob
text [nval=1] ssrname

for i=1...nmark   "The number of SSR markers "
 for j=1...npheno    "The number of phenotype variables"

  conc [tvar] phenoname$[j]     "save the label of the phenotype"
  conc [ssrname] labmark$[i]    "save the label of the ssr marker"
  conc [titl] tvar,', ',labmark$[i]  "make a title for the plots, containing the marker name
and the phenotype label "

  "perform regression of phenotype on the marker, save the Fprobability and the
adjusted r2 "
  model p[j] ; resid=r; fitted=f
  fit [fprob=yes; nome=alias] pssr[i][]

  "Do the checks for normality on the residuals"
  rcheck [title=titl]

  "Save the Fprobability and adjusted r2 from each regression analysis "
  rkeep stat=stat; summ=summ
  calc fprob=1*summ['F pr.']$[1]
  calc adjr2=1*stat$[3]

  prin [ipri=*; squa=yes]tvar, ssrname, fprob, adjr2; fieldwidth=20,20,8,8; deci=*,*,4,2
 endf
endf

stop
```

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## Online Databases:

The ECP/GR Barley Database, Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben: http://barley.ipk-gatersleben.de/ebdb

The GRIN National Plant Germplasm System, United States Department of Agriculture: http://www.ars-grin.gov/npgs/searchgrin.html

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