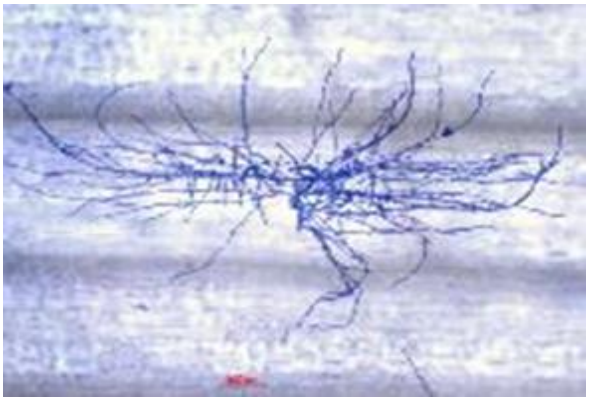
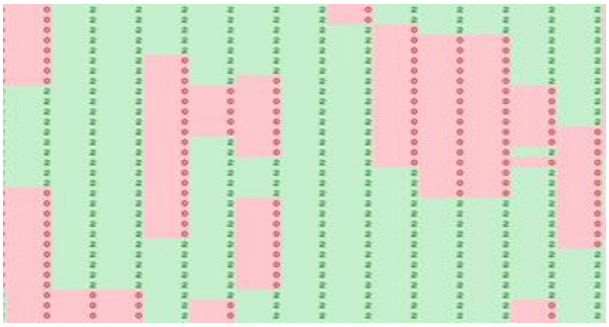
Barley (*Hordeum vulgare* L.) chromosome regions associated with conidiation by powdery mildew fungi in nonhost disease resistance





MSc Thesis

Spencer Matiki

January 2019

WAGENINGEN
UNIVERSITY & RESEARCH

Barley (*Hordeum vulgare* L.) chromosome regions associated with conidiation by powdery mildew fungi in nonhost disease resistance

MSc Thesis (PBR-80436)

Spencer Matiki (861004547130)

Plant Breeding & Genetic Resources

Supervisor and first examiner: Assistant Professor Rients Niks

Second examiner: Professor Yuling Bai

Wageningen University and Research

Laboratory of Plant Breeding

Insect and Nonhost Resistance Group

6708 PB, Wageningen

Droevendaalsesteeg 1 (Radix – gebouw 107)

Information front page: Barley seedlings, wheat mildew colony & chromosome region

Photo courtesy: Spencer Matiki

January 2019

Table of Contents

List of figures

List of tables

Abstract

Nonhost resistance to the non-adapted wheat mildew, *Blumeria graminis* f.sp. *tritici* (*Bgt*) in barley (*Hordeum vulgare* L.) plants would normally result in unsuccessful establishment and conidiation. Such nonhost resistance is effective and durable. Quantitative trait loci (QTLs) for resistance to establishment by *Bgt* were mapped in experimental barley lines. However, the barley QTLs associated with conidiation by wheat mildew are still unknown. We aimed to identify the barley chromosome regions associated with conidiation and explore leaf age/temperature combination that would promote establishment and conidiation by *Bgt*. A subset of recombinant inbred lines (RILs) from Vada x SusBgtDC population was inoculated and evaluated for the level of establishment and conidiation against *Bgt* at the seedling plant stage. Here we show that the barley RILs segregated quantitatively for conidiation. By ordering of RILs and graphical genotyping, we found three chromosome regions associated with conidiation in Vada x SusBgtDC mapping population of barley at the seedling plant stage and were Vada-derived for positive and SusBgtDC-derived for negative conidiation type. The greatest effect was found on chromosome 1H, position 8.6 cM within an interval of 5.7 cM. Leaf age/incubation temperature combination are factors that influence establishment and conidiation by *Bgt* on barley lines. Our results demonstrate that either the parent Vada contribute factors that promote conidiation or parent SusBgtDC prevents conidiation by *Bgt*. We recommend crossing barley lines contrasting for conidiation and fine- mapping studies to identify candidate genes responsible for conidiation by the non-adapted mildew (*Bgt*).

Keywords

Nonhost resistance, *Blumeria graminis* f.sp. *tritici*, chromosome regions, establishment, conidiation

Acknowledgments

This MSc thesis was done at the Plant Breeding Chair Group of Wageningen University & Research (WUR), supervised by Dr. Rients Niks and Dr. Cynara Romero. I am grateful to my daily supervisor and first examiner Dr. Rients Niks for his invaluable help, patience and the trust that I can complete this thesis under his close watch and guidance during my graduate studies. I have nurtured my interest in disease resistance breeding. After Dr. Niks's lecture on Plant Breeding, I knew I had to learn a lot from him, and he gave me the opportunity to work on this project. He was there to clear my doubts in some questions both academically and in other aspects of my personal life which were very useful to me. I also express my gratitude to my second supervisor Dr. Cynara Romero (on distant supervision). She guided me through the report writing and experimental setup. I am very grateful.

I would also like to thank Professor Yuling Bai, my second examiner for her remarkable suggestions and contributions. I am grateful that you took me under your wing in this project.

I am thankful to Anton Vels and Pauline Sanderson, assistants of Unifarm, WUR where I performed all the inoculation experiments. They were willing to provide any information, helpful to my materials and methods. I also thank other workers (WUR) who checked my experiment in the greenhouse compartments.

I express my appreciation to Jasper Vermeulen, Johan Bucher, and technicians of the molecular laboratory for your cooperation and understanding in my laboratory work.

I wish to thank the Netherlands Fellowship Program (NFP) for granting me this scholarship. This is indeed a turning point for me in my career and I look forward to sharing what I have gained to help those in need.

I wish to thank the Allied Timber Zimbabwe (ATZ), a Government Agricultural Parastatal in Zimbabwe under the supervision of Dr. Sithole, for granting me a study leave.

I wish to thank Pastor Farai & Busi Maphosa, Ikeena, Ernest, Elton, Raymond, Onu, Gloriana, technical team and other members of Amazing Grace Parish, Wageningen for their spiritual support.

Finally, innumerable thanks to my family, my wife Abigail and my lovely daughters (Alicia and Adriana), my parents and my sisters. I would not have finished this thesis without their constant support, encouragement and endless love.

1. Introduction

Plants are constantly challenged by pathogen attack. Pathogens deploy specific effectors that impair plant defence system and promote the efficiency of the pathogen to acquire nutrients. Naturally, plants are immobile and cannot evade infection, and have no adaptive immune system (Lee *et al*. 2017). Thus, they make use of varied other defence strategies. Plant's defence against pathogens may be pre-formed as well as induced (Dodds & Rathjen 2010; Eichmann & Hückelhoven 2008). The cell wall and the cuticle are the first line of defence that pathogens face before invading the plant cell (Ferreira *et al*. 2007). Pathogens that overcome the pre-formed defence may trigger the second line of defence, induced barriers (Troch *et al*. 2014). Induced defence is generally classified in two levels of plant-pathogen interaction, one leading to pathogen associated molecular patterns (PAMPs)-triggered immunity (PTI) the other to effector triggered immunity (ETI). PTI postulates the absence of adapted pathogen effectors, leading to a non-compromised plant defence response. Plants possess pattern recognition receptors (PRRs) that recognise the PAMPs leading to a signal that alerts the plant to the presence of a pathogen and confer PTI (Yogendra & Karre 2016). It is difficult for pathogens to alter PAMPs because of fitness penalties. PTI response usually involves ion fluxes, protein phosphorylation and callose deposition (Boller & Felix 2009). The ETI model propose the presence of plant disease resistance genes (*R* genes). In response to pathogen infection, plant's *R* genes encode R proteins, and these R proteins allows the recognition of pathogen effectors. If the effectors encoded by the pathogen avirulence genes (*Avr* genes) matches the target of plant R proteins, these effectors will not cause an infection. ETI is usually characterised by rapid programmed cell death, called hypersensitive response (HR) at the site of infection (Eichmann & Hückelhoven 2008; Lee *et al*. 2016), preventing the spread of infection to the surrounding plant cells.

Adapted pathogens have the appropriate set of effectors to suppress defence in a plant (species), which then acts as a host. If the pathogen effectors do not match the target in a plant, the plant cannot be infected successfully and remains a nonhost. The plant species of which all members are immune to all members of certain potential pathogen species are described as having nonhost resistance (Aghnoum & Niks 2011; Lee *et al*. 2017; Rajaraman *et al*. 2016), and much of the non-host resistance is probably based on PTI. The disease caused by a pathogen may decrease both yield and quality of the crop. Wheat powdery mildew is a major disease of wheat worldwide (Troch *et al*. 2014). Severe infections by wheat powdery mildew on wheat accessions cause substantial yield losses, for example, average grain yield losses due to

Blumeria graminis f.sp. *tritici* (*Bgt*) may exceed 35% in Europe (Delventhal *et al*. 2017). Triazole fungicides are used to control the diseases by powdery mildew fungi, but their use is considered ecologically undesirable (Dean *et al*. 2012; Rsaliyev *et al*. 2017). To reduce agrochemical applications, genetic resistance to plant disease is important in most plant breeding programs (Shtaya *et al*. 2006). Thus, genetic resistance by plants against fungal diseases is a more desirable way to control infections and damage by pathogen species.

Nonhost disease resistance is effective and durable. Therefore, it is important to determine the genetic factors allowing nonhost species to mount resistance against the non-adapted pathogens. Knowledge of such genetic factors is needed to boost the plant system to disease resistance in host species. The study of the genetic basis of nonhost resistance is a challenge, since crosses between plants of different species, a host *versus* a nonhost species, generally result in sterility, hampering genetic analysis. In our laboratory we work on nonhost disease resistance using barley (*Hordeum vulgare* L.) as a model plant species. The diploid nature of barley allows easy genetic and inheritance studies. Barley-powdery mildew fungi as well as barley-rust (Atienza *et al*. 2004; Jafary *et al*. 2006; Schweizer 2007) provide good model pathosystems in which to determine the genetic factors responsible for the non-host status of barley to non-adapted fungi isolates. *Blumeria graminis* is a pathogenic fungus species infecting several plants species of cereals and grasses (Dean *et al*. 2012). Cereal mildew (*Blumeria graminis*) species consist of *formae speciales*, specialised to infect a single host species (Hückelhoven *et al*. 2001). The individual forma specialis (f. sp.) of *Blumeria graminis* exhibit a high degree of host specificity: *Blumeria graminis* f. sp. *hordei* (*Bgh*) infects barley, whereas *Blumeria graminis* f. sp. *tritici* (*Bgt*) infects wheat (*Triticum aestivum*). A number of barley accessions allow some infection by *Blumeria graminis* strains to which barley normally is considered a nonhost. Thus, with that regard to nonhost disease resistance identification of powdery mildew resistance genes at the f.sp level is highly desirable.

Four major components are observed in the life cycle of cereal mildew fungus: spore germination, penetration, haustorium formation, and conidiation. Many of the fungal spores germinate on the leaf surface of the non(host) plants. Successful penetration can be observed on the host plant cell, the adapted mildew fungi successfully penetrate the epidermal cell wall and colonise the leaf surface to form new conidia on conidiophores. On nonhost plant cells, the great majority of penetration attempts by the non-adapted mildew fungi are not successful. So, in nonhost plant species, the non-adapted mildew fungi normally cannot form haustoria because of papilla formation (Chowdhury *et al*. 2016; Niks & Marcel 2009). Such *papilla*-

based resistance prevents the fungus at the penetration stage. However, some barley accessions allow still a bit of establishment by non-adapted fungus (Aghnoum & Niks 2010; Romero *et al*. 2018). Successful establishment of haustoria by a pathogen would allow nutrient uptake by that pathogen and the delivery of effectors into the plant cell. Such infections may grow and develop and are called "established colonies". In barley-*Bgt* interactions, most colonies are only based on one haustorium, and sometimes more than one haustoria is formed, but all in the same cell-and not in the neighbour cells, which cannot support a large colony (Aghnoum & Niks 2010). So, established colonies by *Bgt* on barley cannot develop a large colony, they develop only small colonies, called microcolonies (Romero *et al*. 2018). In barley-*Bgt*, such established microcolonies by *Bgt* may or may not develop further to produce conidia.

Many barley accessions, cultivar (*cv*.) Vada, for example, establishment of even microcolonies by *Bgt* is nil, but on some barley accessions allow *Bgt* infection to a limited extent, and only at the seedling plant stage (Aghnoum & Niks 2010; Romero *et al*. 2018). So, barley accessions with rudimentary susceptibility to *Bgt* were intercrossed in two cycles resulting in two barley lines, called SusBgt lines: SusBgtSC (SC) and SusBgtDC (DC). Aghnoum & Niks (2010) could have selected strongly against barley resistance factors to *Bgt* at the seedling plant stage. Thus, SC and DC lines allowed more *Bgt* infections to establish than their parental lines (Aghnoum & Niks 2010) These two lines, SC and DC are a valuable material to study the genetic basis of nonhost resistance on barley-*Bgt* pathosystem. Romero *et al*. (2018) intercrossed Vada x SC, and Vada x DC to develop two recombinant inbred lines (RILs) mapping populations, that were used for mapping barley quantitative trait loci (QTLs) associated with nonhost resistance to *Bgt* (Romero *et al*. 2018). *Bgt* formed microcolonies about equally successful on SC as on DC. On SC about 20 % of the microcolonies formed conidiophores that could re-infect wheat, but, remarkably, on DC less than 2 % of the microcolonies produced conidia (Romero *et al*. 2018). Therefore, barley genetic factors that determine the establishment of microcolonies by *Bgt* may act independently from genetic factors determining the level of conidiation.

In the study of Romero *et al*. (2018) four QTLs explaining the variation in the establishment of microcolonies were found, one of them important in determining the rate of establishment (Romero *et al*. 2018). This major effect QTL was located on the barley linkage group 5H, explaining more than 40% variance for establishment of microcolonies. Both Vada x SC and Vada x DC showed a similar variation in establishment because of the presence of four QTLs. In both mapping populations, high establishment and low establishment was contributed by parent SusBgt and Vada respectively. In Vada x SC, the RILs that had substantial number of

established microcolonies had almost all also at least some colonies that had formed at least one conidiophore. Although parent DC not (or hardly) allowed conidiation, some RILs from Vada x DC allowed *Bgt* to form conidiophores. Romero *et al*. (2018) hypothesised that some gene contributed by Vada was determinant for increased conidiation rate (Romero *et al*. 2018). Thus, RILs lacking the resistance allele (Vada) of the large-effect QTL for establishment on chromosome 5H could serve to find the chromosome regions that are required for conidiophore formation by *Bgt* in barley. Vada x DC is therefore a suitable mapping population to identify the barley QTLs associated with conidiation by *Bgt*.

The size of effects of QTLs may depend on environmental conditions. We hypothesise that some of the barley RILs carrying the smaller effect QTL at the loci for establishment may allow very few established colonies. The susceptibility of barley accessions to non-adapted powdery mildew is only true at the seedling plant stage, as a younger leaf age could allow more infection by the non-adapted fungal pathogen. Smaller effect QTLs to *Bgt* establishment should be tested by inoculation with *Bgt* spores performed at a younger leaf age and incubation at optimal temperature. Such inoculation at young leaf age and incubation temperature may or may not influence the effect of QTLs promoting or hampering haustorium formation and conidiation by *Bgt* on barley lines. Therefore, the research questions of this study were: (1) Which barley chromosome regions allow conidiation by the non-adapted *Blumeria graminis* f.sp. *tritici*? (2) Can we find the optimal leaf age/incubation temperature for the barley lines with too low establishment of *Bgt* for more reliable data?

2. Materials and methods

2.1 Plant and powdery mildew materials

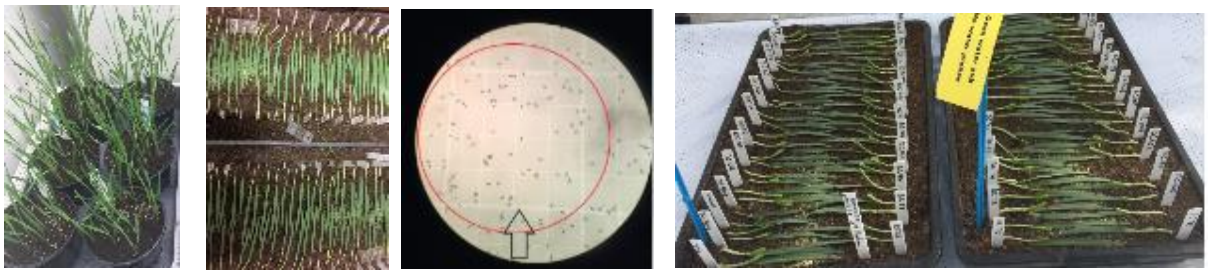
The powdery mildew isolate of wheat (*Bgt*; Swiss field isolate FAL92315) was propagated on wheat *cv.* Vivant (Fig. 1a). Romero *et al.* (2018) described the propagation of wheat powdery mildew (Romero *et al.* 2018). Seeds of *cv.* Vivant and barley lines were obtained from the Laboratory of Plant Breeding (Wageningen University and Research, Wageningen, Netherlands). We used a high-density genetic map of Vada x SusBgtDC that consisted of 115 barley RILs. Rows were sorted according to chromosome and genetic map positions in centimorgans(cM). Forty-five barley RILs showed the SusBgtDC allele of *Rbgqn1* at linkage group 5H, position 143.4 cM (DC_C5-SNP54) as well as the flanking markers. Therefore, barley RILs carrying the SusBgtDC allele that would in principle allow some establishment of *Bgt* microcolonies were grouped (Fig. 2a). Recombinant inbred lines having the Vada allele at locus *Rbgqn1*, preventing establishment, were hidden in the Microsoft excel worksheet (version 2016).

A set of 45 RILs without the Vada resistance allele of the gene *Rbgqn1* was grown on compost soil, as a substrate. We used two plastic trays each measuring 40cm x 60cm. Each tray contained 22 or 23 barley RILs and the parental lines (barley *cv.* Vada, SusBgtDC), reference lines (SusBgtSC and wheat *cv.* Vivant). Each barley RIL, the parental, and the reference lines in each tray was represented by two seedlings. The *cv.* Vada and SusBgtDC served as negative controls whereas SusBgtSC and *cv.* Vivant as positive controls for conidiation by *Bgt*. Barley plants were grown in a greenhouse compartment at incubation temperature, 20 °C day-time and 16 °C night-time, 40-60% relative humidity, 16h photoperiod in a greenhouse compartment until they were 13-days-old as previously described (Romero *et al.* 2018). The sulphur was kept on preventing conditions (plant physiology) that would promote/stimulate development of powdery mildew. Lamps were kept at a height of *c.* 2.1m, thus low light intensity on the seedling leaf surface. The whole experiment consisted of three consecutive replications and was conducted at the Unifarm greenhouse facilities of Wageningen University and Research, Netherlands.

2.2 Inoculation of barley leaves with powdery mildew isolate *Bgt*

Aghnoum & Niks (2010) described the procedure of preparing the *Bgt* inoculum and performing the inoculations on barley seedlings. In the current study we performed inoculations by *Bgt* on 13-day old seedlings of barley RILs, parental lines and reference lines. For each seedling, we removed all young leaves and kept the first leaf. The first leaf of each seedling

was pinned horizontally to the substrate with the adaxial side up, using metal pins. The leaves of the seedlings were randomly spread over the two sides of a tray. Leaf segments of *cv*. Vivant (*c*.5cm) infected by *Bgt* were harvested from the host plants propagated in a growth cabinet and put in two square petri dishes, each measuring 12.5cm x 12.5cm. The petri dishes were carried to the inoculation room with its lid. Three haemocytometers were placed on top of the trays to check spore (*Bgt*) density on leaf samples (Fig. 1b). Inoculations were performed in a settling box measuring 100cm x 120cm x 87cm, where the two trays containing the barley lines were placed on the bottom of the settling box and to be inoculated simultaneously with *Bgt* isolate. Fresh conidia from heavily sporulating host leaf (*cv*. Vivant) segments were blown into the settling box using compressed air. The compressed air was blown for 15 seconds against the walls of the inoculation chamber to prevent strong turbulence of leaves but allowing uniform spread of *Bgt* spores. Spore density was checked 10 minutes after spore application. We aimed at average *Bgt* spore density of *c.* 20 to 30 conidia/mm^2 leaf area (Fig. 1c). If there is no enough spore density more *Bgt* infected material were gathered and blown as previously described. Inoculated seedlings were then transferred to a second greenhouse compartment with same growth conditions as previously described (Section 2.1). The leaf surface of the inoculated seedlings was not watered, and the metal pins were kept on the leaves for 24hrs (Fig. 1d). We kept the inoculated seedlings until the moment of harvesting the inoculated leaf segments for microscopic evaluation: seven days after inoculation.



a **b** **c** **d**

Fig. 1 Summary of inoculation experiment (a) Wheat mildew maintenance on cultivar Vivant (b) Three haemocytometer to check spore density (c) Counting spores under light microscope, *c.* 20-30 microspores/mm^2 (d) 24 hrs after inoculation of barley lines with *Blumeria graminis* f.sp. *tritici*.

2.3 Bleaching, sectioning and staining of leaf segments

For each seedling per RIL, parental and reference line, we collected a leaf segment of *c.* 6 cm that was harvested from the middle section of the inoculated leaves. Two leaf segments were collected per line per experiment. Leaf segments were then transferred to a solution of acetic-96% ethanol (1:3 v/v) and bleached for at least 24 hours. We optimised the protocol suggested

by Wolf & Fric (1981) by decreasing the concentration of Coomassie Brilliant Blue (CBB) (Wolf & Fric 1981), resulting in the following treatment: solution 15% trichloroacetic acid (TCA) and 0.075% CBB in 99% methanol (w/v). Staining solution modified from Wolf & Fric (1981) consisted of decreased concentration CBB and increased staining time. This allowed the best visualisation of the stained leaf segments. Each harvested leaf segment was cut into two halves. Leaf segments with their adaxial side down were immersed for 60 mins in the staining solution in a petri dish. Stained leaf segments were then transferred to a solution of acetic acid-glycerol-Milli-Q water (5:20:75) and cleared for at least 10 minutes to remove the excess dye. Leaf segments were mounted on microscopic slides by embedding the stained leaf segments in 100% glycerol, with the adaxial side up for light microscopy (Wolf & Fric 1981). The first object slide contained one half segment of both seedlings, and the second object slide for both seedlings the second half of the sampled segment.

2.4 Microscopic evaluation and scoring of barley lines-*Blumeria graminis* f.sp. *tritici* interaction

We analysed all the leaf samples using the bright field microscopy with a total magnification of 10x ocular and 10x objective under a white light microscope. Microscopic analysis was done for 44 barley RILs, parental and reference lines. We observed a blue-black coloration on the cellular components of mildew (*Bgt*) due to the accumulation of staining solution. We counted the total number of established microcolonies per RIL and then determined the proportion of the established microcolonies forming conidia. RILs allowing the establishment of at least 20 microcolonies for all analysed leaf samples were considered as informative.

The proportion of established microcolonies forming at least one conidiophore were named reproductive established colonies (REC) and the average number of conidiophores per REC was determined. We ordered the data of barley RILs, for the RILs positive for conidiation from many to few conidia, and for the RILs negative for conidiation from many establishment to few establishment. Statistical analyses were performed using GenStat (VSN International 2018). An ANOVA followed by a Fisher's protected LSD ($P < 0.05$) was performed to test for significant differences in the rates of establishment and formation of conidia.

2.5 Graphical genotyping for barley recombinant inbred lines from Vada x SusBgtDC mapping population to conidiation

Graphical genotyping is a visually attractive and easily interpretable method to identify loci associated with a certain phenotype. Application of filters to select a subset of single nucleotide

polymorphisms (SNPs) allows one to visualise haplotype sharing between individuals that also share a specific phenotype. SNP data will then merge into an image which displays a distal genomic region on the arm of a certain chromosome where a specific haplotype, in this study, represented a haplotype of barley lines carrying a gene(s) preventing or allowing conidiation. So, graphical genotyping was relevant in our study of finding barley chromosome regions associated with conidiation by *Bgt*. For graphical genotypes, we used 44 barley RILs from Vada x SusBgtDC population, allowing the establishment of *Bgt* microcolonies and simultaneously checking for the presence or absence of the SNPs associated with conidiation by *Bgt.*

Filter and ordering were applied on the Microsoft excel worksheet that consisted of 44 barley RILs, previously described (Section 2.4). The scores on the rate of establishment and conidiation by *Bgt* on barley RILs were used to order these 44 RILs from highest to lowest reliability. We counted all established colonies on the whole leaf area, and, in case one leaf area had >20 we stopped after reaching the 50. In this study, the barley lines that give the most reliable information were lines that showed >20 established micro colonies in each of the three replications (category one). The next reliable give >20 in some but not all replications (category two), followed by those in each replication <20, but accumulated total number was 20 over the three replications (category three). Least informative if even over the three replications less than 20 established micro colonies were found (category four) (Table 1 a, b). Lines with enough established microcolonies (>20) were grouped into two, positive conidiation and negative conidiation type. Barley RILs were then ordered from many to few conidia for positive conidiation, whereas many established to few established colonies for negative conidiation type. In the high-density map of Vada x SusBgtDC mapping population, chromosome regions of these 44 barley RILs were compared to find the loci that agreed for positive and negative conidiation. The alleles found in the SNPs marker surrounding the loci (<5cM) associated with conidiation were determined.

2.6 Inoculation at seedling leaf age and incubation temperature stimulating the establishment and conidiation by *Blumeria graminis* f.sp. *tritici*

We hypothesise that leaf age and temperature affect the establishment and conidiation by *Bgt* on barley seedling plants. So, we set up an experiment to explore leaf age and temperature for which establishment would be highest. We used four trays of size 38 cm x 25 cm sown with four barley accessions, three plants per accession and in one inoculation experiment. Two barley lines, SusBgtSC and RIL DC-23 that showed conidiation were selected together with

two lines SusBgtDC and RIL DC-105 that did not show conidiation. These four lines allowed many established colonies at leaf age (13-day-old) and incubation temperature (20/16 °C). Two lines DC and SC, extra three seedlings were sown three days later in the same tray to have younger leaf age tested for DC and SC. After, seven days at 20/16 °C temperature (day/night), trays were transfer to four temperature regimes: 20/18 °C, 22/20 °C, 23/20 °C, and 25/21 °C for three days. We then switched off the sulphur to possibly promote and or condition the plant physiology for the development of powdery mildew. In each greenhouse compartment lamps were lowered closer to the tray (*c.* 1.2m) to allow more light (high light intensity) on barley seedlings. We inoculated the trays with *Bgt* at once and density of *c.* 20 to 30 conidia per mm^2 was aimed. Then, trays were returned to the four respective regimes, until the moment of harvesting leaf segments: seven days after inoculation. Bleaching and subsequent procedures were performed as described in section 2.3 and 2.4.

Statistical analyses were performed on phenotypic evaluations using GenStat (VSN International 2018). An ANOVA followed by a Fisher's protected LSD ($P < 0.05$) was performed to test for significant differences in the rates of establishment and formation of conidia at four temperature regimes. We only analysed for the effect of leaf age in the establishment and conidiation by *Bgt* on the two barley lines: SusBgtSC and SusBgtDC. We then performed a Student t-test ($P < 0.05$) using GenStat software to test for significance difference in establishment of colonies and conidiation at leaf age (10- and 13-day-old).

We set up a second experiment to use the age and temperature that were most conducive for establishment and conidiation to test four barley lines that before had too low establishment to conclude conidiation potential. We used leaf age (six and nine days) and incubation temperature (22/20 °C). Three seedlings per line were grown, in two consecutive inoculation experiments. Subsequent experiment procedures were performed as previously described (Section 2.2, 2.3, and 2.4)

3. Results

3.1 Phenotyping of the conidiation to the non-adapted *Blumeria graminis* f.sp. *tritici*

Forty-five RILs from Vada x SusBgtDC barley mapping population together with parental lines, and reference lines (SusBgtSC and wheat *cv*. Vivant) were evaluated for the presence (positive) or absence (negative) of reproductive colonies (RECs) by *Blumeria graminis* f.sp. *tritici*. One RIL: DC-101, was excluded in the phenotypic analysis due to too few seedlings, and the few seedlings gave too few established colonies to allow reliable classification for positive or negative conidiophore formation. In each replication, at leaf age (13-day-old) and incubation temperature of 20/16°C (day/night), *cv*. Vivant showed macroscopic signs of infection by *Bgt*. Parent SusBgtDC and reference line (SusBgtSC), and other RILs showed macroscopically only a low number of established microcolonies. No conidiation was observed on SusBgtDC leaf samples, at the time of inoculation (13-day-old), kept at incubation temperature (20/16 °C). Vada did not show any established microcolonies. Nineteen out of 44 RILs (43.2%) the establishment rate by *Bgt* was so high that in each replication over 20 established colonies were found per two leaves per line, classified as establishment category one (Table 1 a; b). Surprisingly, few barley RILs, nine out of 44 RILs (20.5%) allowed formation of conidia (Table 1a), we expected about 50% of the 44 RILs to show conidiation. On RILs on which *Bgt* establishes at high rates, the conidiation was not necessarily high. Thirty-five out of the 44 RILs (79.5%) did not show any RECs. On 31 RILs there were at least 20 established colonies in total over three replications (establishment category one to three). However, four RILs showed too few established colonies to allow reliable classification for positive or negative conidiophore formation (Table 1b) (shaded region: orange)

A similar score in the average number of 10-11 established microcolonies/cm^2 was observed on parent SusBgtDC and reference line (SusBgtSC) and almost all RILs had fewer established colonies than SusBgtDC parent (Table 1 a, b). We observed some (small) differences in the establishment frequency by *Bgt* between the most convincing REC RIL (many established colonies and many conidia) and non-REC RIL (many established colonies and no conidia). This was clearly illustrated by RILs DC-23 and DC-105: DC-23 showed RECs whereas no RECs were observed on DC-105. No significant difference in established microcolonies was observed between RIL DC-23 (9.88 microcolonies/cm^2) and RIL DC-105 (8.77 microcolonies/cm^2) (Table 1 a, b). Therefore, RIL DC-105 serve as a more convincingly negative for RECs (high establishment but negative for conidiation) than in RILs with establishment category two, three or four.

Table 1. Microscopic scores from the interaction of *Blumeria graminis* f.sp. *tritici* with a subset of *Hordeum vulgare* (barley) recombinant inbred lines (RILs) from Vada x SusBgtDC population, including parents and SusBgtSC. The scores represent average data of three replicate inoculation experiments, with two leaf segments per experiment per line. Average scores in the number of established microcolonies and conidiation rate (a) barley RILs positive for conidiation (b) barley RILs negative for conidiation (unshaded region). In each column, scores sharing the same letter are not significantly different ($P < 0.05$). Letters start in both tables separately.

a)

Line	Est cat[1]	Tot.Est	Col EF (cm^2)[2]	% Conid (REC)	Conid/ REC	Genotype (REC_EST)
SusBgtSC	1	327	10.0 a	9.7 a	5.0 a	SSS _SSS
DC-23	1	298	9.88 a	6.6 ab	4.0 a	VVV_SSS
DC-22	1	290	9.83 a	6.0 bc	3.8 a	VVV_SSV
DC-115	1	240	6.70 b	4.4 bcd	2.1 b	VVV_SVV
DC-111	1	231	6.62 b	3.7 bcde	1.8 b	VVV_SVV
DC-70	1	200	6.50 b	3.2 cde	1.5 b	VVV_SSV
DC-87	2	196	3.44 c	2.3 de	1.5 b	VVV_VSV
DC-44	1	194	6.40 b	1.6 de	1.2 b	VVS_SSV
DC-02	1	181	6.16 b	1.2 e	1.0 b	VVV_SSV
DC-13	1	175	6.13 b	1.0 e	1.0 b	VSV_SVS

b)

Line	Est cat[1]	Tot.Est	Col EF (cm^2)[2]	% Conid (REC)	Conid/REC	Genotype (REC_EST)
SusBgtDC	1	304	11.0 a	0	-	SSS_SSS
DC-105	1	285	8.77 b	0	-	SSS_SVV
DC-81	1	244	7.14 bc	0	-	SSS_SVS
DC-102	1	237	6.81 bcd	0	-	SSS_SVS
DC-84	1	205	6.07 cde	0	-	SSS_SVS
DC-106	1	167	5.14 de	0	-	SSS_SVS
DC-54	1	134	4.17 ef	0	-	SSS_SVS
DC-78	1	114	4.15 ef	0	-	SSS_VVS
DC-74	2	99	3.14 f	0	-	SVS_SVS
DC-47[3]	1	97	2.88 f	0	-	SVV_VSS
DC-104[3]	1	86	2.76 f	0	-	SVV_SSS
DC-27[3]	1	80	2.73 f	0	-	SVV_VSS
Vada	4	0	-	-	-	VVV_VVV
DC-109[4]	4	14	0.39 mno	-	-	VVV_VSV
DC-73[4]	4	12	0.29 no	-	-	SSV_VVS
DC-20[4]	4	2	0.07 o	-	-	SVV_SVS
DC-17[4]	4	1	0.03 o	-	-	VSS_SSV

[1]Establishment category
1 = > 20 established microcolonies in each rep (n=3), 2 = in 1 or 2 reps established microcolonies > 20, 3 = no rep > 20, but total over reps > 20, and 4 = total over reps < 20

[2]EF = Establishment frequency microcolonies per cm^2

[3]Barley lines positive for Vada allele but showed no RECs at linkage group 6HA and 6HB

[4]RILs that showed too few established colonies and absence of conidiation by *Bgt*, not distinguished to either positive or negative conidiation group (Shaded orange)

In each table the barley lines were ordered from the most convincing RIL to least convincing RIL.Parental lines are represented: V for Vada, S for SusBgtDC. Reference line, SusBgtSC (S). For each RIL REC genotype (1H, 6HA and 6HB) and minor QTLs for establishment (2H, 4H and 1H)

Some differences in the rate of RECs and the number of conidiophores per REC was observed between the RILs. This is well illustrated by RILs DC-23 and DC-13: the rate of RECs differed significantly from 6.6% (four conidiophores per REC) in the former to 1% (one conidiophore per REC) in the second (Table 1a). RIL DC-87 (establishment category two) had 3.44 established microcolonies/cm^2 that differed significantly from establishment scores of all other eight REC RILs (Table 1a). The rate of conidiation observed on RIL DC-87 did not differ significantly to the least convincing REC RIL DC-13, that showed too few conidia.

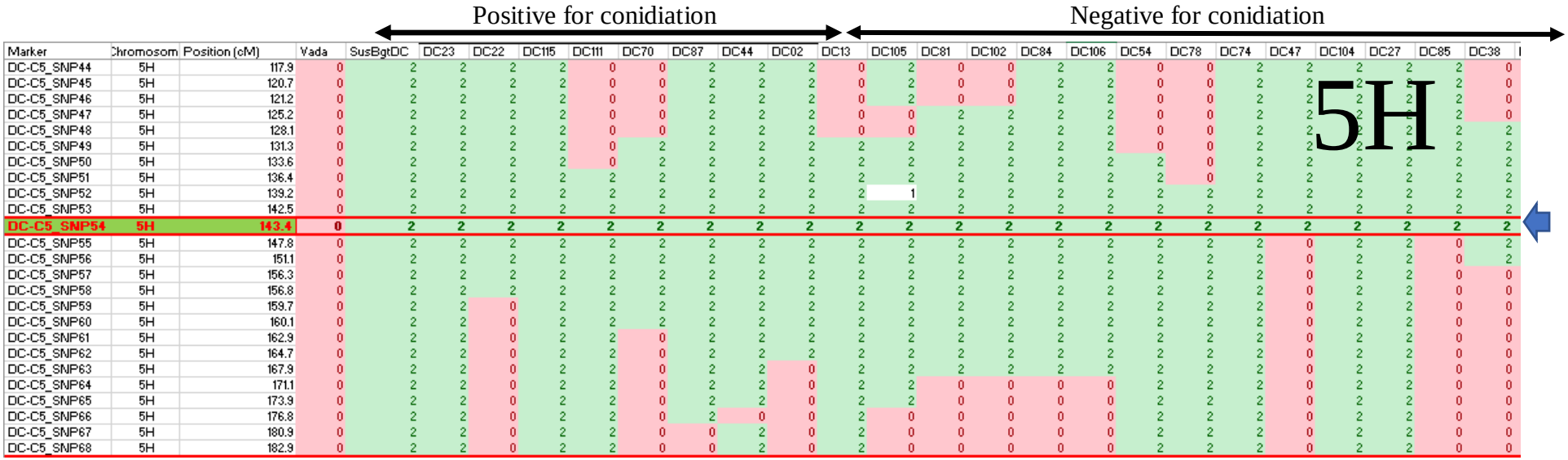
3.2 Graphical genotyping using the high-density and skeletal maps of Vada x SusBgtDC

The detailed map of Vada x SusBgtDC shows the positions where the conidiation gene may be found. High-density genetic map of Vada x SusBgtDC consisted a total of 7,422 SNP markers, with largest gap between two adjacent loci of 8.8 cM on linkage group 6H (from 11.9 cM to 20.7 cM), some markers were found on the same chromosome position on 1H (0 cM interval) and the total genetic length was 1023 cM (Romero *et al*. 2018). In the skeletal map those data are lost. So, the skeletal map is good to show the overall picture over the whole barley genome, but the places that are of interest are better defined from the detailed full-marker set picture. The skeletal map of Vada x SusBgtDC consisted a total of 354 SNPs markers. We set the function of 'VLOOKUP' in excel worksheet (Microsoft Excel 2016) and imported the data of SNPs markers from the high-density to skeletal map of Vada x SusBgtDC mapping population. In each conidiation group (positive and negative), we ranked the barley RILs from the most convincing to less convincing, positive (many conidia to few conidia) and negative (many established to few established colonies). All 44 barley RILs had the SusBgtDC allele of 5H (Fig. 2a). On SNP markers that are not associated with conidiation, chromosome regions carried either an allele of Vada or SusBgtDC parent (Fig. 2b). We found three regions on barley chromosomes associated with reproductive colonies ('REC' region) by *Bgt* (Fig. 2 c, f) in the high-density map, on chromosomes 1H (Fig. 2 c, d, e) and 6H (Fig. 2f). At these three regions on the barley chromosomes, about nine of the most convincing REC RILs have the Vada allele of the marker, and nearly all of the most convincing non-REC RILs had the SusBgtDC allele (Table 1 a, b). There was no region that was uniformly SusBgtDC for the positive conidiation group and uniformly Vada for the negative conidiation. The region to promote conidiation was from parent Vada, consistent with Romero *et al*. (2018) hypothesis of parent Vada carrying the genes that allowed conidiation (Romero *et al*. 2018). Parent SusBgtDC contributed the factor that prevents/reduces conidiation. It seems either Vada or SusBgtDC have the active factor for the conidiation loci.

We then visualised the loci associated with conidiation in the skeletal map. These three REC regions were found on two linkage groups: 1H and 6H, linkage group 6H showed two REC regions and were named: 6HA and 6HB from the most convincing to less convincing (Fig. 2f). Eight out of nine RIL (88.9%) that showed REC have the allele of Vada at all three linkage groups for conidiation (Table 1a), and these eight RILs (nearly) all had the SusBgtDC allele of QTL *Rbgqn2* for establishment (Fig. 2g). Three RILs of the most convincing non-REC RILs showed the Vada allele of QTLs (6HA and 6HB) (Table 1b, Fig. 2f). These three RILs had SusBgtDC allele on QTL (1H) for conidiation (Table S3). A marker significantly associated with conidiation was found on chromosome 1H at position 8.6 cM of interval 5.7 cM (from 5.3 cM to 11 cM).

Some differences in the conidiation rate was observed between the barley RILs that carried similar alleles at the REC loci. The difference in the number of RECs between RILs positive for conidiation could be due to absence of Vada allele at the conidiation loci on some linkage group. This is well illustrated by RILs DC-22 and DC-13: their genotype VVV and VSV respectively. RIL DC-22 had the Vada allele at all linkage group. RIL DC13 was the least convincing REC RIL and had the Vada allele at chromosome 1H and 6HB, but on linkage group 1H the SusBgtDC alleles were found in the surrounding markers (< 3.3 cM) (Fig. 2c) and on chromosome 6HB one of the flanking regions carried the SusBgtDC allele (Fig. 2f). RIL DC-44 and DC-13 had the SusBgtDC allele and even in the surrounding markers on 6HB and 6HA respectively. RIL DC-44 and DC-13 were found in the lower convincing REC RILs.

Much of the RILs negative for conidiation have SusBgtDC allele at three linkage groups for conidiation (SSS) (Fig. 2 c,e,f) and have SusBgtDC allele of QTL (*Rbgqn2*) for establishment, this explains the many establishment and absence of RECs. Some barley RILs are phenotypically contrasting, but differ only for the linkage group 1H for REC. This is well represented by DC-23 (**V**VV_SSS) and DC-104 (**S**VV_SSS), both lines have Vada allele of QTLs (6HA and 6HB) for conidiation, DC-104 showed no RECs in contrast to the most convincing REC RIL DC-23. The absence of Vada allele of linkage group 1H could explain non-REC observed on RIL DC-104. Although we did not carry QTL analysis, the strongest effect of the three QTL could be 1H > 6HA = or > 6HB. We could not classify RILs with establishment category four. The RIL DC-109 of establishment category four, carried a similar genotype to RIL DC-87 for the positive conidiation type, whereas RIL DC-20 of establishment category four only differed on QTL(*Rbgqn3*), minor QTL for establishment to RIL DC-104 of negative conidiation type. Both DC-109 and DC-20 had too few established colonies.

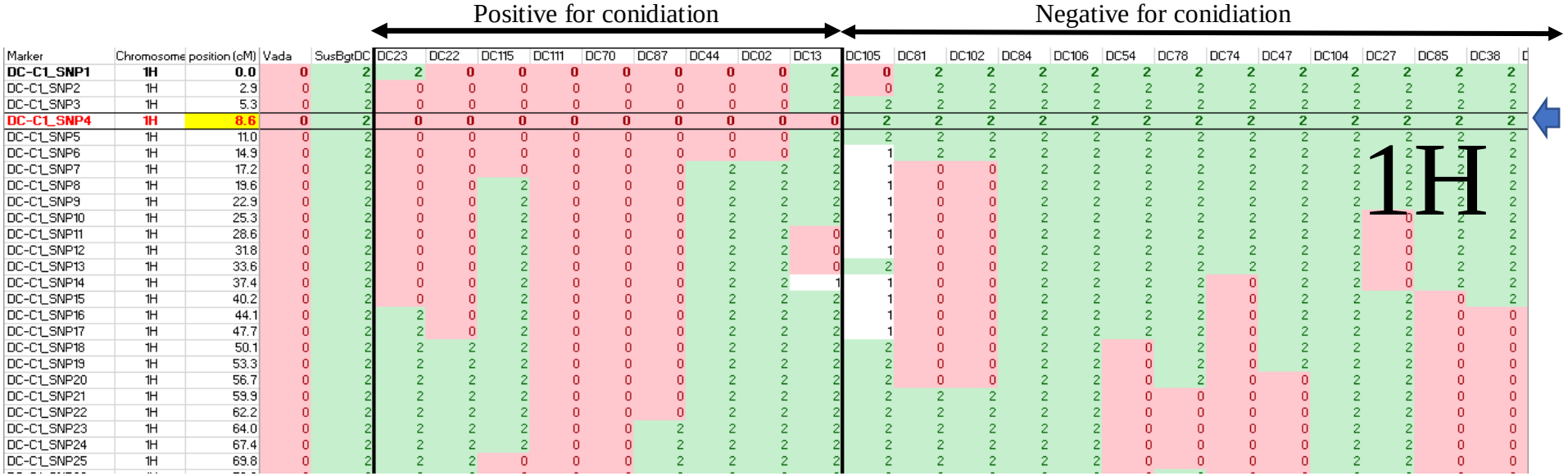


Marker	Chromosom	Position (cM)	Vada	SusBgtDC	DC23	DC22	DC115	DC111	DC70	DC87	DC44	DC02	DC13	DC105	DC81	DC102	DC84	DC106	DC54	DC78	DC74	DC47	DC104	DC27	DC85	DC38
DC-C5_SNP44	5H	117.9	0	2	2	2	2	0	0	2	2	2	0	2	0	0	2	2	0	0	2	2	2	2	2	0
DC-C5_SNP45	5H	120.7	0	2	2	2	2	0	0	2	2	2	0	2	0	0	2	2	0	0	2	2	2	2	2	0
DC-C5_SNP46	5H	121.2	0	2	2	2	2	0	0	2	2	2	0	2	0	0	2	2	0	0	2	2	2	2	2	0
DC-C5_SNP47	5H	125.2	0	2	2	2	2	0	0	2	2	2	0	0	2	2	2	2	0	0	2	2	2	2	2	0
DC-C5_SNP48	5H	128.1	0	2	2	2	2	0	0	2	2	2	0	0	2	2	2	2	0	0	2	2	2	2	2	2
DC-C5_SNP49	5H	131.3	0	2	2	2	2	0	2	2	2	2	2	2	2	2	2	2	0	0	2	2	2	2	2	2
DC-C5_SNP50	5H	133.6	0	2	2	2	2	0	2	2	2	2	2	2	2	2	2	2	2	0	2	2	2	2	2	2
DC-C5_SNP51	5H	136.4	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0	2	2	2	2	2	2
DC-C5_SNP52	5H	139.2	0	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2
DC-C5_SNP53	5H	142.5	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
DC-C5_SNP54	**5H**	**143.4**	**0**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
DC-C5_SNP55	5H	147.8	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0	2	2	0	2
DC-C5_SNP56	5H	151.1	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0	2	2	0	2
DC-C5_SNP57	5H	156.3	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0	2	2	0	0
DC-C5_SNP58	5H	156.8	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0	2	2	0	0
DC-C5_SNP59	5H	159.7	0	2	2	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0	2	2	0	0
DC-C5_SNP60	5H	160.1	0	2	2	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0	2	2	0	0
DC-C5_SNP61	5H	162.9	0	2	2	0	2	2	0	2	2	2	2	2	2	2	2	2	2	2	2	0	2	2	0	0
DC-C5_SNP62	5H	164.7	0	2	2	0	2	2	0	2	2	2	2	2	2	2	2	2	2	2	2	0	2	2	0	0
DC-C5_SNP63	5H	167.9	0	2	2	0	2	2	0	2	2	0	2	2	2	2	2	2	2	2	2	0	2	2	0	0
DC-C5_SNP64	5H	171.1	0	2	2	0	2	2	0	2	2	0	2	2	0	0	0	0	2	2	2	0	2	2	0	0
DC-C5_SNP65	5H	173.9	0	2	2	0	2	2	0	2	2	0	2	2	0	0	0	0	2	2	2	0	2	2	0	0
DC-C5_SNP66	5H	176.8	0	2	2	0	2	2	0	2	0	0	2	0	0	0	0	0	2	2	2	0	2	2	0	0
DC-C5_SNP67	5H	180.9	0	2	2	0	2	2	0	0	2	0	2	0	0	0	0	0	2	2	2	0	2	2	0	0
DC-C5_SNP68	5H	182.9	0	2	2	0	2	2	0	0	2	0	2	0	0	0	0	0	2	2	2	0	2	2	0	0

Fig. 2a Barley RILs were selected with the SusBgtDC allele for high establishment on 5H, position 143.4cM (*Rbgqn1*). Red=Vada,Green = SusBgtDC

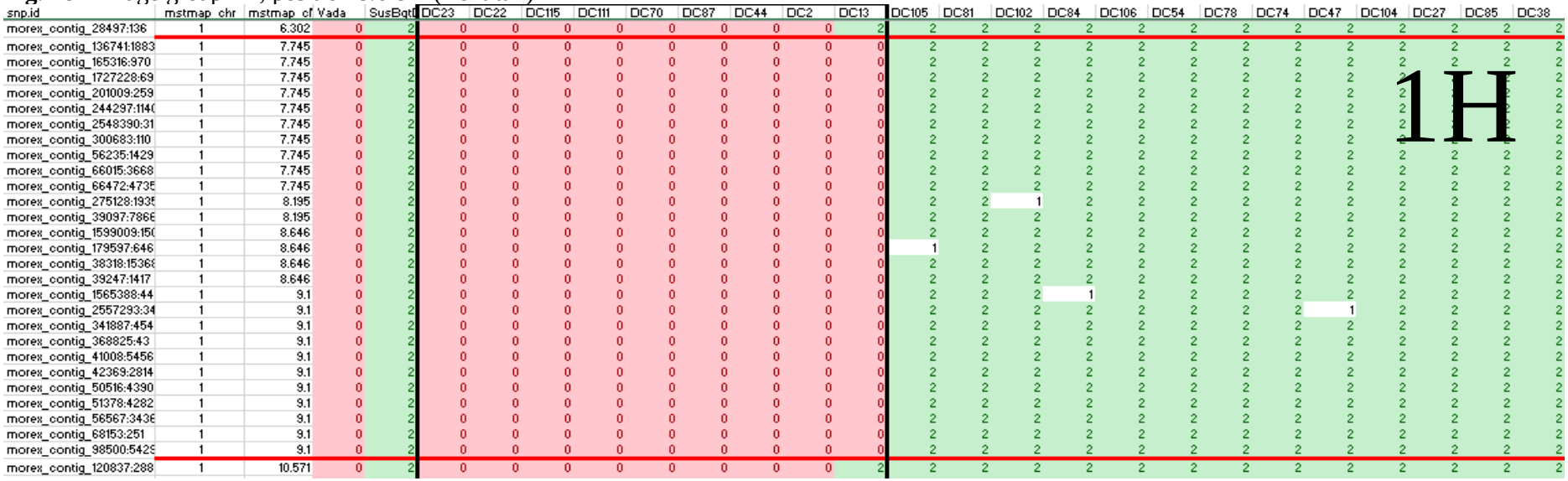
Marker	Chromosom	Position (cM)	Vada	SusBgtDC	DC23	DC22	DC115	DC111	DC70	DC87	DC44	DC02	DC13	DC105	DC81	DC102	DC84	DC106	DC54	DC78	DC74	DC47	DC104	DC27	DC85	DC38
DC-C6_SNP38	6H	106.2	0	2	0	0	0	2	2	2	2	2	0	0	2	2	0	0	2	2	0	2	0	2	0	0
DC-C7_SNP1	7H	0.0	0	2	2	2	0	0	0	0	2	2	2	2	0	0	2	2	2	0	2	2	1	2	2	2
DC-C7_SNP2	7H	3.2	0	2	2	2	0	0	0	0	2	2	2	2	0	0	2	2	2	0	2	2	0	2	2	2
DC-C7_SNP3	7H	6.3	0	2	2	2	2	0	0	0	2	2	2	0	0	0	2	2	2	0	2	2	0	2	2	2
DC-C7_SNP4	7H	9.2	0	2	2	2	2	0	0	0	2	2	2	0	0	0	2	2	2	2	2	2	0	2	2	2
DC-C7_SNP5	7H	12.0	0	2	0	2	2	0	0	0	2	2	2	0	2	0	2	2	2	2	2	2	0	2	2	2
DC-C7_SNP6	7H	14.9	0	2	0	2	2	0	0	0	2	2	2	0	2	0	2	2	2	2	2	0	0	2	0	2
DC-C7_SNP7	7H	17.2	0	2	0	2	2	0	0	0	0	2	2	0	2	0	2	2	2	2	2	0	0	2	0	0
DC-C7_SNP8	7H	20.5	0	2	2	2	2	0	0	0	0	2	2	0	2	0	2	2	2	0	2	0	0	2	0	0
DC-C7_SNP9	7H	23.3	0	2	2	2	2	0	0	0	0	2	2	0	2	0	2	2	2	0	2	0	0	2	0	0
DC-C7_SNP10	7H	26.6	0	2	2	2	2	2	0	0	0	2	2	0	2	0	2	2	2	0	2	0	0	2	0	0
DC-C7_SNP11	7H	29.0	0	2	2	2	2	2	0	0	0	2	0	0	2	0	2	2	2	0	2	0	0	2	0	0
DC-C7_SNP12	7H	32.6	0	2	2	2	2	2	0	2	0	2	0	0	2	0	2	2	2	0	2	0	0	2	0	2
DC-C7_SNP13	7H	34.9	0	2	2	2	2	2	0	2	0	0	0	0	0	0	2	2	2	0	2	0	0	2	0	2
DC-C7_SNP14	7H	37.6	0	2	0	2	2	2	0	0	0	0	0	0	0	0	2	2	2	0	2	0	0	2	0	2
DC-C7_SNP15	7H	39.5	0	2	0	2	2	2	0	0	0	0	0	0	0	0	2	2	2	0	2	0	0	2	0	2
DC-C7_SNP16	7H	42.3	0	2	0	2	2	2	0	0	2	0	2	0	0	0	2	2	2	0	2	0	0	2	0	2
DC-C7_SNP17	7H	45.2	0	2	0	2	2	2	2	0	2	0	0	0	0	0	2	2	0	0	2	0	0	2	0	2
DC-C7_SNP18	7H	47.4	0	2	0	2	2	2	2	0	2	0	0	0	0	0	2	2	0	0	2	0	0	2	2	2
DC-C7_SNP19	7H	50.7	0	2	0	2	2	2	2	0	2	0	0	0	0	0	2	2	0	0	2	0	0	2	2	0
DC-C7_SNP20	7H	52.1	0	2	0	0	2	2	2	0	2	0	0	0	0	0	2	2	0	0	2	0	0	2	2	0
DC-C7_SNP21	7H	54.5	0	2	0	2	2	2	2	0	2	0	0	0	2	2	2	2	0	0	2	0	0	2	2	0
DC-C7_SNP22	7H	56.8	0	2	0	2	2	2	2	0	2	0	0	0	2	2	2	2	0	0	2	0	0	2	2	0
DC-C7_SNP23	7H	59.5	0	2	0	2	2	2	2	0	2	0	0	0	2	2	2	2	0	0	2	0	0	0	2	0
DC-C7_SNP24	7H	62.5	0	2	0	2	2	2	2	0	2	0	0	0	2	2	2	2	0	0	2	0	0	0	2	0

Fig. 2b Random linkage group that is not associated with conidiation (7H). Red = Vada, Green = SusBgtDC



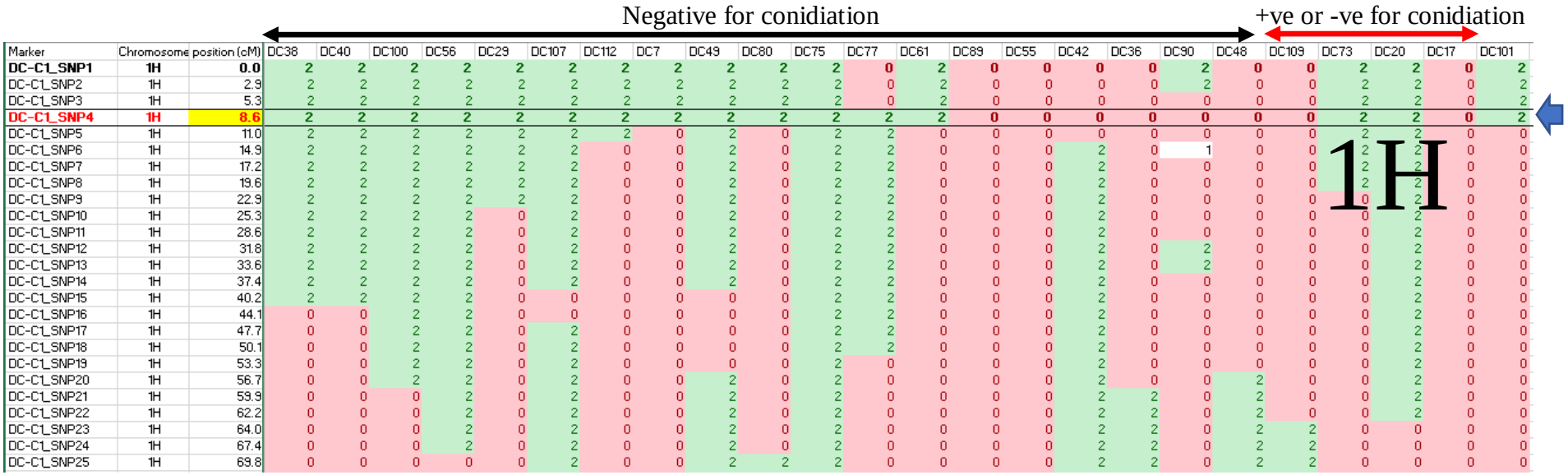
Marker	Chromosome	position (cM)	Vada	SusBgtDC	DC23	DC22	DC115	DC111	DC70	DC87	DC44	DC02	DC13	DC105	DC81	DC102	DC84	DC106	DC54	DC78	DC74	DC47	DC104	DC27	DC85	DC38
DC-C1_SNP1	**1H**	**0.0**	**0**	**2**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
DC-C1_SNP2	1H	2.9	0	2	0	0	0	0	0	0	0	0	2	0	2	2	2	2	2	2	2	2	2	2	2	2
DC-C1_SNP3	1H	5.3	0	2	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2
DC-C1_SNP4	**1H**	**8.6**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
DC-C1_SNP5	1H	11.0	0	2	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2
DC-C1_SNP6	1H	14.9	0	2	0	0	0	0	0	0	0	0	2	1	2	2	2	2	2	2	2	2	2	2	2	2
DC-C1_SNP7	1H	17.2	0	2	0	0	0	0	0	0	2	2	2	1	0	0	2	2	2	2	2	2	2	2	2	2
DC-C1_SNP8	1H	19.6	0	2	0	0	2	0	0	0	2	2	2	1	0	0	2	2	2	2	2	2	2	2	2	2
DC-C1_SNP9	1H	22.9	0	2	0	0	2	0	0	0	2	2	2	1	0	0	2	2	2	2	2	2	2	2	2	2
DC-C1_SNP10	1H	25.3	0	2	0	0	2	0	0	0	2	2	2	1	0	0	2	2	2	2	2	2	2	2	2	2
DC-C1_SNP11	1H	28.6	0	2	0	0	2	0	0	0	2	2	0	1	0	0	2	2	2	2	2	2	2	0	2	2
DC-C1_SNP12	1H	31.8	0	2	0	0	2	0	0	0	2	2	0	1	0	0	2	2	2	2	2	2	2	0	2	2
DC-C1_SNP13	1H	33.6	0	2	0	0	2	0	0	0	2	2	0	2	0	0	2	2	2	2	2	2	2	0	2	2
DC-C1_SNP14	1H	37.4	0	2	0	0	2	0	0	0	2	2	1	1	0	0	2	2	2	2	0	2	2	0	2	2
DC-C1_SNP15	1H	40.2	0	2	0	0	2	0	0	0	2	2	2	1	0	0	2	2	2	2	0	2	2	2	0	2
DC-C1_SNP16	1H	44.1	0	2	2	0	2	0	0	0	2	2	2	1	0	0	2	2	2	2	0	2	2	2	0	0
DC-C1_SNP17	1H	47.7	0	2	2	0	2	0	0	0	2	2	2	1	0	0	2	2	2	2	0	2	2	2	0	0
DC-C1_SNP18	1H	50.1	0	2	2	2	2	0	0	0	2	2	2	2	0	0	2	2	0	2	0	2	2	2	0	0
DC-C1_SNP19	1H	53.3	0	2	2	2	2	0	0	0	2	2	2	2	0	0	2	2	0	2	0	2	2	2	0	0
DC-C1_SNP20	1H	56.7	0	2	2	2	2	0	0	0	2	2	2	2	0	0	2	2	0	2	0	0	2	2	0	0
DC-C1_SNP21	1H	59.9	0	2	2	2	2	0	0	0	2	2	2	2	2	2	2	2	0	0	0	0	2	2	0	0
DC-C1_SNP22	1H	62.2	0	2	2	2	2	0	0	0	2	2	2	2	2	2	2	2	0	0	0	0	2	2	0	0
DC-C1_SNP23	1H	64.0	0	2	2	2	2	0	0	2	2	2	2	2	2	2	2	2	0	0	0	0	2	2	0	0
DC-C1_SNP24	1H	67.4	0	2	2	2	2	0	0	2	2	2	2	2	2	2	2	2	0	0	0	0	2	2	0	0
DC-C1_SNP25	1H	69.8	0	2	2	2	0	0	0	2	2	2	2	2	2	2	2	2	0	0	0	0	2	2	0	0

Fig. 2c Linkage group 1H, position 8.6 cM (front tail)



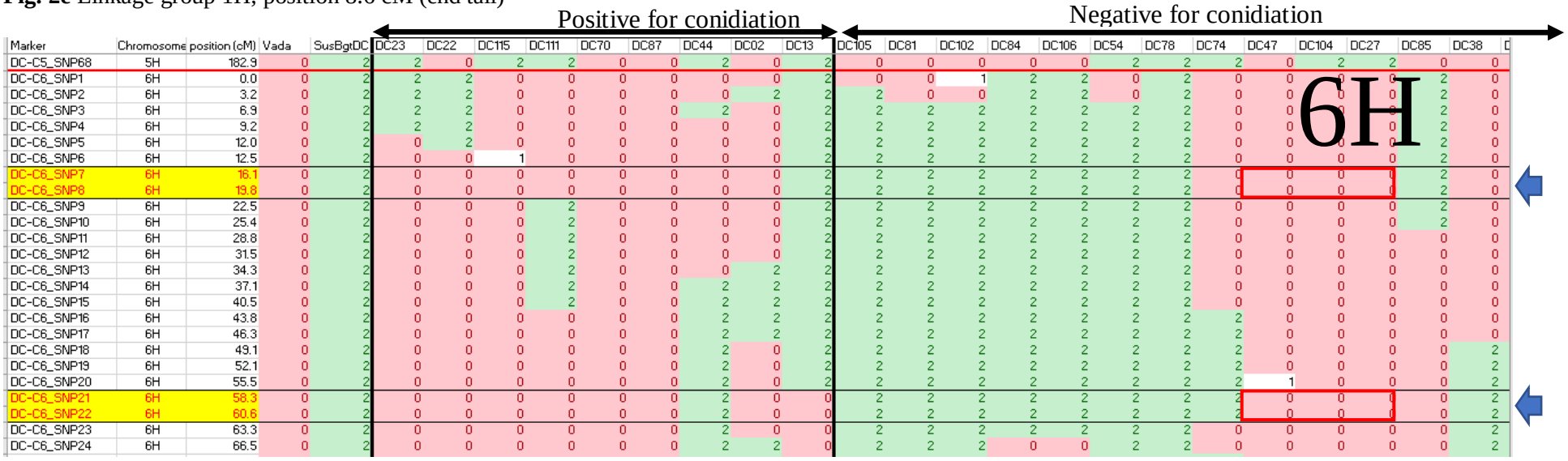
snp.id	mstmap_chr	mstmap_cM	Vada	SusBgtDC	DC23	DC22	DC115	DC111	DC70	DC87	DC44	DC2	DC13	DC105	DC81	DC102	DC84	DC106	DC54	DC78	DC74	DC47	DC104	DC27	DC85	DC38
morex_contig_28497:136	1	6.302	0	2	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_136741:1883	1	7.745	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_165316:970	1	7.745	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_1727228:69	1	7.745	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_201009:259	1	7.745	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_244297:1140	1	7.745	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_2548390:31	1	7.745	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_300683:110	1	7.745	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_56235:1429	1	7.745	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_66015:3668	1	7.745	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_66472:4735	1	7.745	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_275128:1935	1	8.195	0	2	0	0	0	0	0	0	0	0	0	2	2	1	2	2	2	2	2	2	2	2	2	2
morex_contig_39097:7866	1	8.195	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_1599009:150	1	8.646	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_179597:646	1	8.646	0	2	0	0	0	0	0	0	0	0	0	1	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_38318:1536	1	8.646	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_39247:1417	1	8.646	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_1565388:44	1	9.1	0	2	0	0	0	0	0	0	0	0	0	2	2	2	1	2	2	2	2	2	2	2	2	2
morex_contig_2557293:34	1	9.1	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	1	2	2	2	2
morex_contig_341887:454	1	9.1	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_368825:43	1	9.1	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_41008:5456	1	9.1	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_42369:2814	1	9.1	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_50516:4390	1	9.1	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_51378:4282	1	9.1	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_56567:3436	1	9.1	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_68153:251	1	9.1	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_98500:5429	1	9.1	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
morex_contig_120837:288	1	10.571	0	2	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2

Fig. 2d Linkage group 1H, position 8.6 cM (high-density map, more details). Red=Vada, Green=SusBgtDC



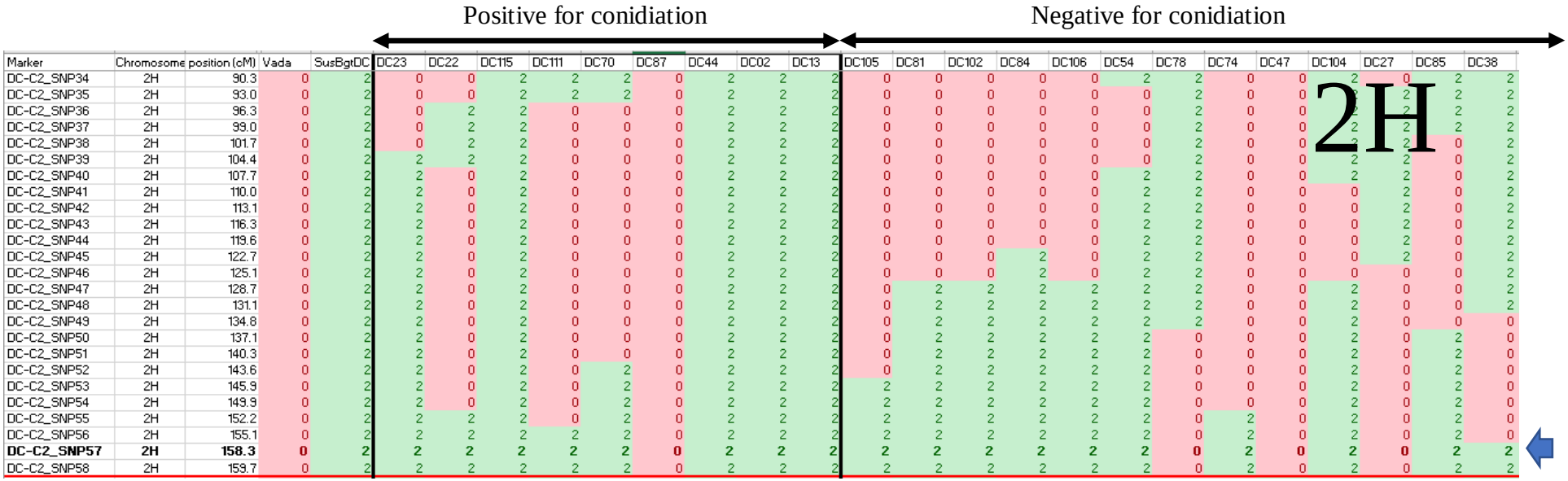
Marker	Chromosome	position (cM)	DC38	DC40	DC100	DC56	DC29	DC107	DC112	DC7	DC49	DC80	DC75	DC77	DC61	DC89	DC55	DC42	DC36	DC90	DC48	DC109	DC73	DC20	DC17	DC101
DC-C1_SNP1	**1H**	**0.0**	2	2	2	2	2	2	2	2	2	2	2	0	2	0	0	0	0	2	0	0	2	2	0	2
DC-C1_SNP2	1H	2.9	2	2	2	2	2	2	2	2	2	2	2	0	2	0	0	0	0	2	0	0	2	2	0	2
DC-C1_SNP3	1H	5.3	2	2	2	2	2	2	2	2	2	2	2	0	2	0	0	0	0	0	0	0	2	2	0	2
DC-C1_SNP4	**1H**	**8.6**	2	2	2	2	2	2	2	2	2	2	2	2	2	0	0	0	0	0	0	0	2	2	0	2
DC-C1_SNP5	1H	11.0	2	2	2	2	2	2	2	0	2	0	2	2	0	0	0	0	0	0	0	0	2	2	0	0
DC-C1_SNP6	1H	14.9	2	2	2	2	2	2	0	0	2	0	2	2	0	0	0	2	0	1	0	0	2	2	0	0
DC-C1_SNP7	1H	17.2	2	2	2	2	2	2	0	0	2	0	2	2	0	0	0	2	0	0	0	0	2	2	0	0
DC-C1_SNP8	1H	19.6	2	2	2	2	2	2	0	0	2	0	2	2	0	0	0	2	0	0	0	0	2	2	0	0
DC-C1_SNP9	1H	22.9	2	2	2	2	2	2	0	0	2	0	2	2	0	0	0	2	0	0	0	0	2	2	0	0
DC-C1_SNP10	1H	25.3	2	2	2	2	0	2	0	0	2	0	2	2	0	0	0	2	0	0	0	0	0	2	0	0
DC-C1_SNP11	1H	28.6	2	2	2	2	0	2	0	0	2	0	2	2	0	0	0	2	0	0	0	0	0	2	0	0
DC-C1_SNP12	1H	31.8	2	2	2	2	0	2	0	0	2	0	2	2	0	0	0	2	0	2	0	0	0	2	0	0
DC-C1_SNP13	1H	33.6	2	2	2	2	0	2	0	0	2	0	2	2	0	0	0	2	0	2	0	0	0	2	0	0
DC-C1_SNP14	1H	37.4	2	2	2	2	0	2	0	0	2	0	2	2	0	0	0	2	0	0	0	0	0	2	0	0
DC-C1_SNP15	1H	40.2	2	2	2	2	0	0	0	0	0	0	2	2	0	0	0	2	0	0	0	0	0	2	0	0
DC-C1_SNP16	1H	44.1	0	0	2	2	0	0	0	0	0	0	2	2	0	0	0	2	0	0	0	0	0	2	0	0
DC-C1_SNP17	1H	47.7	0	0	2	2	0	2	0	0	0	0	2	2	0	0	0	2	0	0	0	0	0	2	0	0
DC-C1_SNP18	1H	50.1	0	0	2	2	0	2	0	0	0	0	2	2	0	0	0	2	0	0	0	0	0	2	0	0
DC-C1_SNP19	1H	53.3	0	0	2	2	0	2	0	0	0	0	2	0	0	0	0	2	0	0	0	0	0	2	0	0
DC-C1_SNP20	1H	56.7	0	0	2	2	0	2	0	0	2	0	2	0	0	0	0	2	0	0	2	0	0	2	0	0
DC-C1_SNP21	1H	59.9	0	0	0	2	0	2	0	0	2	0	2	0	0	0	0	2	2	0	2	0	0	2	0	0
DC-C1_SNP22	1H	62.2	0	0	0	2	0	2	0	0	2	0	2	0	0	0	0	2	2	0	2	0	0	2	0	0
DC-C1_SNP23	1H	64.0	0	0	0	2	0	2	0	0	2	0	2	0	0	0	0	2	2	0	2	2	0	0	0	0
DC-C1_SNP24	1H	67.4	0	0	0	2	0	2	0	0	2	0	2	0	0	0	0	2	2	0	2	2	0	0	0	0
DC-C1_SNP25	1H	69.8	0	0	0	0	0	2	0	0	2	2	2	0	0	0	0	2	2	0	2	2	0	0	0	0

Fig. 2e Linkage group 1H, position 8.6 cM (end tail)



Marker	Chromosome	position (cM)	Vada	SusBgtDC	DC23	DC22	DC115	DC111	DC70	DC87	DC44	DC02	DC13	DC105	DC81	DC102	DC84	DC106	DC54	DC78	DC74	DC47	DC104	DC27	DC85	DC38
DC-C5_SNP68	5H	182.9	0	2	2	0	2	2	0	0	2	0	2	0	0	0	0	0	2	2	2	0	2	2	0	0
DC-C6_SNP1	6H	0.0	0	2	2	2	0	0	0	0	0	0	2	0	0	1	2	2	0	2	0	0	0	0	2	0
DC-C6_SNP2	6H	3.2	0	2	2	2	0	0	0	0	0	2	2	2	0	0	2	2	0	2	0	0	0	0	2	0
DC-C6_SNP3	6H	6.9	0	2	2	2	0	0	0	0	2	0	2	2	2	2	2	2	2	2	0	0	0	0	2	0
DC-C6_SNP4	6H	9.2	0	2	2	2	0	0	0	0	0	0	2	2	2	2	2	2	2	2	0	0	0	0	2	0
DC-C6_SNP5	6H	12.0	0	2	0	2	0	0	0	0	0	0	2	2	2	2	2	2	2	2	0	0	0	0	2	0
DC-C6_SNP6	6H	12.5	0	2	0	0	1	0	0	0	0	0	2	2	2	2	2	2	2	2	0	0	0	0	2	0
DC-C6_SNP7	6H	16.1	0	2	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	0	0	0	0	2	0
DC-C6_SNP8	6H	19.8	0	2	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	0	0	0	0	2	0
DC-C6_SNP9	6H	22.5	0	2	0	0	0	2	0	0	0	0	2	2	2	2	2	2	2	2	0	0	0	0	2	0
DC-C6_SNP10	6H	25.4	0	2	0	0	0	2	0	0	0	0	2	2	2	2	2	2	2	2	0	0	0	0	2	0
DC-C6_SNP11	6H	28.8	0	2	0	0	0	2	0	0	0	0	2	2	2	2	2	2	2	2	0	0	0	0	0	0
DC-C6_SNP12	6H	31.5	0	2	0	0	0	2	0	0	0	0	2	2	2	2	2	2	2	2	0	0	0	0	0	0
DC-C6_SNP13	6H	34.3	0	2	0	0	0	2	0	0	0	2	2	2	2	2	2	2	2	2	0	0	0	0	0	0
DC-C6_SNP14	6H	37.1	0	2	0	0	0	2	0	0	2	2	2	2	2	2	2	2	2	2	0	0	0	0	0	0
DC-C6_SNP15	6H	40.5	0	2	0	0	0	2	0	0	2	2	2	2	2	2	2	2	2	2	0	0	0	0	0	0
DC-C6_SNP16	6H	43.8	0	2	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	0	0	0	0	0
DC-C6_SNP17	6H	46.3	0	2	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	0	0	0	0	0
DC-C6_SNP18	6H	49.1	0	2	0	0	0	0	0	0	2	0	2	2	2	2	2	2	2	2	2	0	0	0	0	2
DC-C6_SNP19	6H	52.1	0	2	0	0	0	0	0	0	2	0	2	2	2	2	2	2	2	2	2	0	0	0	0	2
DC-C6_SNP20	6H	55.5	0	2	0	0	0	0	0	0	2	0	2	2	2	2	2	2	2	2	2	1	0	0	0	2
DC-C6_SNP21	6H	58.3	0	2	0	0	0	0	0	0	2	0	0	2	2	2	2	2	2	2	2	0	0	0	0	2
DC-C6_SNP22	6H	60.6	0	2	0	0	0	0	0	0	2	0	0	2	2	2	2	2	2	2	2	0	0	0	0	2
DC-C6_SNP23	6H	63.3	0	2	0	0	0	0	0	0	2	0	0	2	2	2	2	2	2	2	0	0	0	0	0	2
DC-C6_SNP24	6H	66.5	0	2	0	0	0	0	0	0	2	2	0	2	2	2	0	0	2	2	0	0	0	0	0	2

Fig. 2f Linkage group 6H. Two regions 6HA and 6HB at the positions 16.1 cM and 58.3 cM respectively



Marker	Chromosome	position (cM)	Vada	SusBgtDC	DC23	DC22	DC115	DC111	DC70	DC87	DC44	DC02	DC13	DC105	DC81	DC102	DC84	DC106	DC54	DC78	DC74	DC47	DC104	DC27	DC85	DC38
DC-C2_SNP34	2H	90.3	0	2	0	0	2	2	2	0	2	2	2	0	0	0	0	0	2	2	0	0	2	0	2	2
DC-C2_SNP35	2H	93.0	0	2	0	0	2	2	2	0	2	2	2	0	0	0	0	0	0	2	0	0	2	2	2	2
DC-C2_SNP36	2H	96.3	0	2	0	2	2	0	0	0	2	2	2	0	0	0	0	0	0	2	0	0	2	2	2	2
DC-C2_SNP37	2H	99.0	0	2	0	2	2	0	0	0	2	2	2	0	0	0	0	0	0	2	0	0	2	2	2	2
DC-C2_SNP38	2H	101.7	0	2	0	2	2	0	0	0	2	2	2	0	0	0	0	0	0	2	0	0	2	2	0	2
DC-C2_SNP39	2H	104.4	0	2	2	2	2	0	0	0	2	2	2	0	0	0	0	0	0	2	0	0	2	2	0	2
DC-C2_SNP40	2H	107.7	0	2	2	0	2	0	0	0	2	2	2	0	0	0	0	0	2	2	0	0	2	2	0	2
DC-C2_SNP41	2H	110.0	0	2	2	0	2	0	0	0	2	2	2	0	0	0	0	0	2	2	0	0	0	2	0	2
DC-C2_SNP42	2H	113.1	0	2	2	0	2	0	0	0	2	2	2	0	0	0	0	0	2	2	0	0	0	2	0	2
DC-C2_SNP43	2H	116.3	0	2	2	0	2	0	0	0	2	2	2	0	0	0	0	0	2	2	0	0	0	2	0	2
DC-C2_SNP44	2H	119.6	0	2	2	0	2	0	0	0	2	2	2	0	0	0	0	0	2	2	0	0	0	2	0	2
DC-C2_SNP45	2H	122.7	0	2	2	0	2	0	0	0	2	2	2	0	0	0	2	0	2	2	0	0	0	2	0	2
DC-C2_SNP46	2H	125.1	0	2	2	0	2	0	0	0	2	2	2	0	0	0	2	0	2	2	0	0	0	0	0	2
DC-C2_SNP47	2H	128.7	0	2	2	0	2	0	0	0	2	2	2	0	2	2	2	2	2	2	0	0	2	0	0	2
DC-C2_SNP48	2H	131.1	0	2	2	0	2	0	0	0	2	2	2	0	2	2	2	2	2	2	0	0	2	0	0	2
DC-C2_SNP49	2H	134.8	0	2	2	0	2	0	0	0	2	2	2	0	2	2	2	2	2	2	0	0	2	0	0	0
DC-C2_SNP50	2H	137.1	0	2	2	0	2	0	0	0	2	2	2	0	2	2	2	2	2	0	0	0	2	0	2	0
DC-C2_SNP51	2H	140.3	0	2	2	0	2	0	0	0	2	2	2	0	2	2	2	2	2	0	0	0	2	0	2	0
DC-C2_SNP52	2H	143.6	0	2	2	0	2	0	2	0	2	2	2	0	2	2	2	2	2	0	0	0	2	0	2	0
DC-C2_SNP53	2H	145.9	0	2	2	0	2	0	2	0	2	2	2	2	2	2	2	2	2	0	0	0	2	0	2	0
DC-C2_SNP54	2H	149.9	0	2	2	0	2	0	2	0	2	2	2	2	2	2	2	2	2	2	0	0	0	2	0	2	0
DC-C2_SNP55	2H	152.2	0	2	2	2	2	0	2	0	2	2	2	2	2	2	2	2	2	2	0	2	0	2	0	2	0
DC-C2_SNP56	2H	155.1	0	2	2	2	2	2	2	0	2	2	2	2	2	2	2	2	2	2	0	2	0	2	0	2	0
DC-C2_SNP57	**2H**	**158.3**	**0**	**2**	**2**	**2**	**2**	**2**	**2**	**0**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**0**	**2**	**0**	**2**	**0**	**2**	**2**
DC-C2_SNP58	2H	159.7	0	2	2	2	2	2	2	0	2	2	2	2	2	2	2	2	2	0	2	0	2	0	2	2

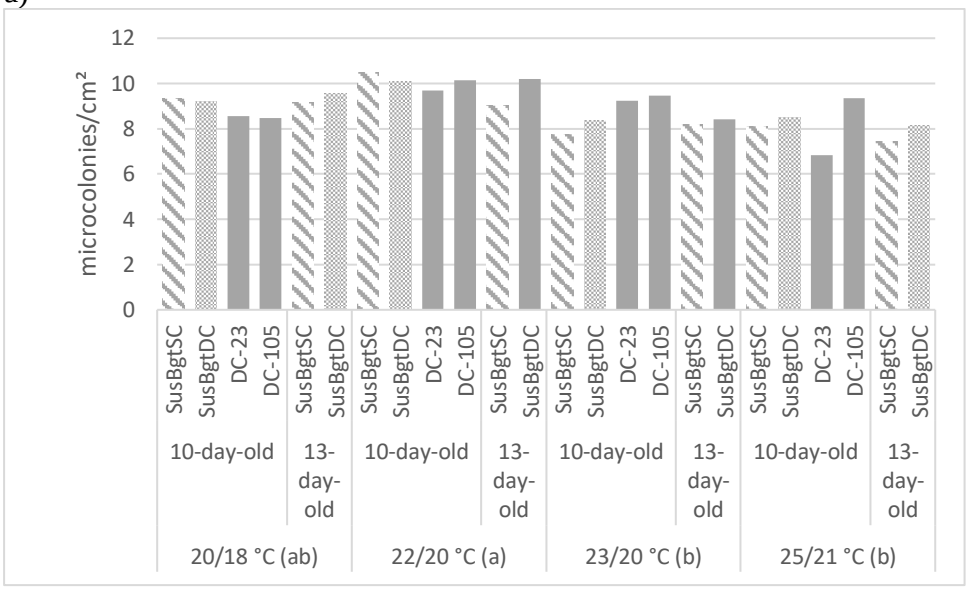
Fig. 2g Minor QTL for establishment on linkage group 2H (*Rbgqn2*) at position 158.3 cM

Fig. 2 Single nucleotide polymorphisms (SNPs) markers in the Vada x SusBgtDC skeletal map. The skeletal map represent data of 44 barley RILs. Shaded columns along each linkage group represent the allele of the donor parent, red = Vada; green = SusBgtDC. Rows along each linkage group represent the name of the SNP marker and the position in centimorgans (cM). In each figure from RIL DC-23 to RIL DC-13 were positive (vertical bold black) and from RIL DC-105 to RIL DC-38 negative for conidiation. Horizontal bold red line shows the start or end of each linkage group (a) Region selected against the immune allele of Vada on DC-C5_SNP54, the peak marker for microcolony establishment: *Rbgnq1*.In each group positive and negative for conidiation, barley RILs were ordered from the most convincing to least convincing. Random linkage group 7H (a). Barley chromosome regions associated with conidiation by *Bgt*: c, d, e (1H), (f) 6HA and 6HB, (g) Minor QTL for establishment on 2H (*Rbgqn2*)

3.3 Seedling leaf age at inoculation and incubation temperature are factors that influence the establishment and conidium formation by *Bgt* on barley.

We hypothesised that inoculation at the seedling leaf age influence the establishment by *Bgt* in the barley RILs from Vada x SusBgtDC population. The different incubation temperatures resulted in differences in the establishment rate between treatments (Fig. 3a). Reference line (SusBgtSC) allowed substantial conidiation at each incubation temperature. Surprisingly, the parent SusBgtDC (that hardly formed any conidia at 13-day old/ 20/16°C) showed substantial reproductive colonies by *Bgt* at two incubation temperatures: 20/18°C and 22/20°C (day/night) (Fig. 3b). This suggests that parent SusBgtDC have factors that allow more RECs under certain conditions. However, SusBgtSC allowed more conidia than SusBgtDC. We ignored the effects of individual genotypes and aimed at the effect of temperature at inoculation for statistical analysis. Leaf age and temperature interaction was not significant. Three barley lines at 23/20°C and 25/21°C scored significantly lower in the rate of RECs than at 22/20°C (Fig. 3b). The rate of conidiation on 10-day-old leaves scored not significantly lower than samples of leaves inoculated with *Bgt* at 13-day-old. The effect of leaf age and temperature on establishment rate was not significantly different between the treatments. High light intensity and sulphur could have contributed some factor(s) that prevent/ promote establishment and conidiation by *Bgt*. Therefore, the optimal temperature at incubation that result in highest establishment and highest REC was 22/20°C.

a)



b)

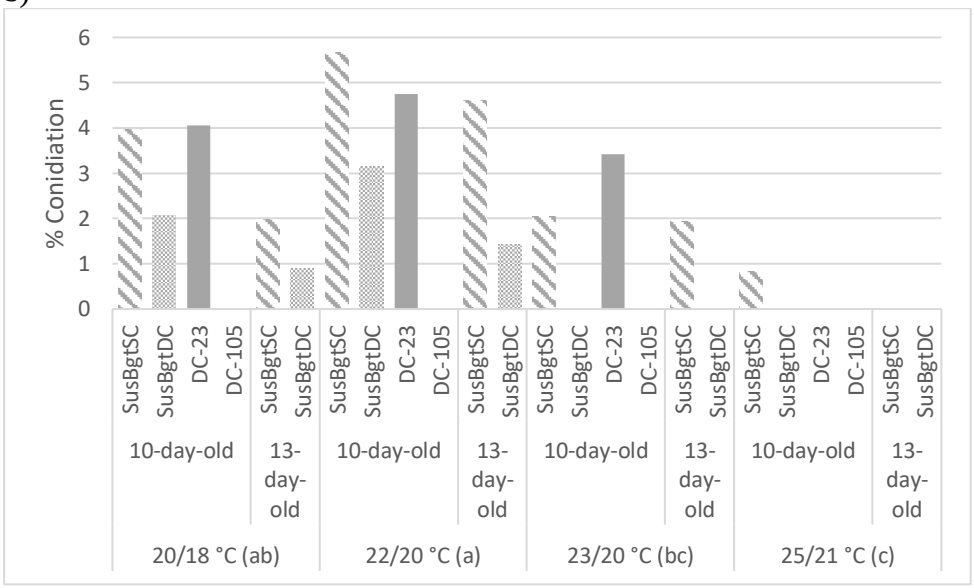


Fig. 3 Microscopic scores from the interaction of *Blumeria graminis* f.sp. *tritici* with a subset of recombinant inbred lines from Vada x SusBgtDC mapping population, including the parents. Four incubation temperature and two seedling leaf ages were compared. The bars represent average data of one experiment, with four leaf segments per incubation temperature. Barley lines are represented by pattern bars: diagonal stripes for SusBgtSC, dotted for SusBgtDC and grey for other RILs (a) Average score in the number of established microcolonies/cm^2 counted under the light microscope seven days after inoculation with *Bgt*. (b) Conidiation rate: percentage of reproductive colonies seven days after inoculation with *Bgt*. Incubation temperature sharing the same letter (in brackets) are not significantly different ($P < 0.05$)

3.4 Barley recombinant inbred lines shows too few established colonies at some incubation temperature

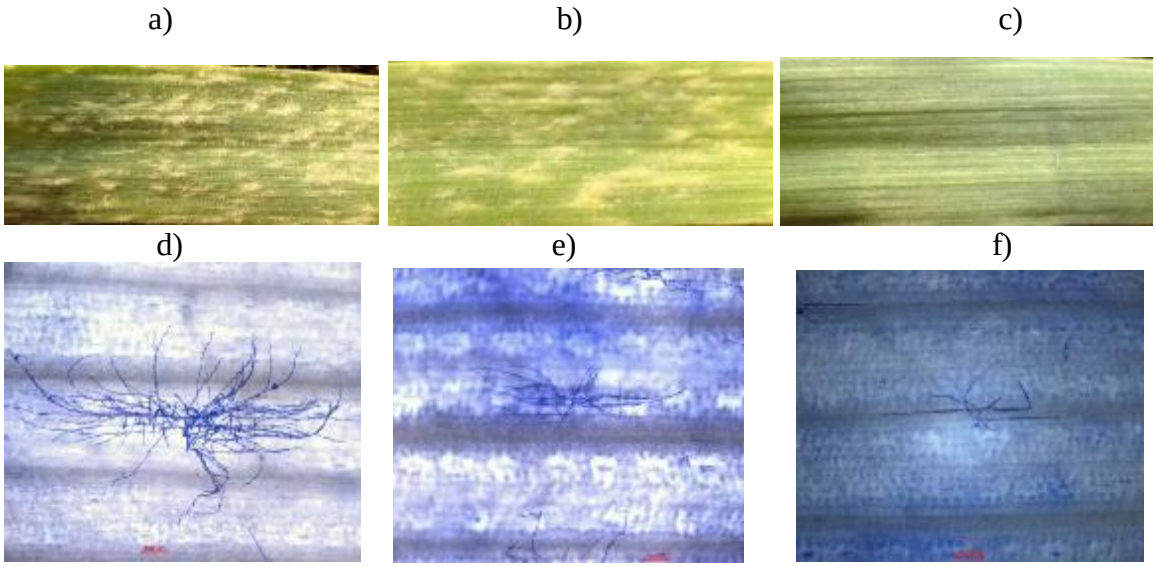


Fig. 4 Infection units of *Blumeria graminis* f.sp. *tritici* (*Bgt*) on barley (*Hordeum vulgare*) plants, six days after inoculation. The size of colonies on SusBgtSC and SusBgtDC was in two consecutive experiments (22/20°C) much larger than in previous three inoculation experiments (20/16°C). Inoculation was performed at seedling leaf age of six day and incubation temperature of 22/20 °C. Macroscopic signs of infection by *Bgt*: (a) SusBgtSC and (b) SusBgtDC, (c) no visible signs of infection on RIL DC-20. Microcolonies by *Bgt* on barley lines: (d) Substantial conidiophore formation on SusBgtSC (e) SusBgtDC (f) less developed colony on RIL DC-20.

Some of the low establishment category RILs could be classified for conidiation more reliably by offering optimal temperature conditions. Highest establishment by *Bgt* was observed at the incubation temperature (22/20°C). We presume that macroscopic signs of infection on SusBgtSC and SusBgtDC looked like *Bgt* on wheat (Fig. 4 a, b). Five days after rubbing the SusBgtSC and SusBgtDC infections, on wheat *cv*.Vivant abundant mildew infection occurred but not on barley SusPtrit, indicating that the high rate of infection on SusBgtSC and SusBgtDC was due to *Bgt* and not to *Bgh*. Few macroscopic signs of infection were observed on RIL DC-20 (Fig. 4c). Reference line, SusBgtSC had a higher score in the number of RECs as well as a higher average in the number (> 5) of conidiophores per REC (Fig. 4d) than parent SusBgtDC. Large colonies of *Bgt* were merged and these large colonies (probably) formed more than one haustorium and were successful in extracting enough nutrients to support a large colony. This implies that the selected temperature regime on some barley lines, promoted the establishment of microcolonies by *Bgt*. The establishment by *Bgt* was high at 22/20°C between RILs that showed too few established colonies at 20/16 °C (Table 1b, shaded orange), but four RILs still could not be classified either positive or negative for conidiation (< 20 over two reps).

4. Discussion and Conclusions

Nonhost disease resistance is effective and durable, plant breeders exploit such nonhost resistance to improve disease resistance within host species. Thus, knowledge of molecular basis of nonhost status of plants to potential pathogens is needed to mimic the pseudo-nonhost resistance in a host plant species. We presume that such disease resistance would be effective and durable in barley lines against the adapted mildew pathogen.

Nonhost resistance in barley to *Blumeria graminis* f.sp. *tritici* (*Bgt*) prevents establishment. However, some experimental barley lines (SusBgtSC and SusBgtDC) developed with atypically high establishment by the non-adapted pathogen were crossed with Vada, and the mapping population segregated quantitatively, Vada contributing the genes against such establishment by *Bgt*. We selected against the Vada immunity allele of the major quantitative trait loci for establishment (*Rbgqn1*) in the Vada x SusBgtDC population, to avoid the barley RILs that would not allow enough *Bgt* establishment. This was done in order to distinguish positive conidiation types from negative conidiation types.

Our results showed that inoculations at leaf age (six day old), incubation temperature of 22/20 °C and high light intensity contribute to establishment and conidiation by *Bgt* on some Vada x SusBgtDC RILs (Table S2). *Bgt* seems to be more developed than in our earlier inoculation experiments (leaf age 13-day-old, temperature 20/16 °C), SC and DC showed macroscopic signs of infection four days after inoculation and many of the *Bgt* colonies were merged (Fig. S1). The level of conidiation found on SC/DC-*Bgt* is still lower than during a compatible wheat-*Bgt* interaction. However, factors such as leaf age, incubation and light intensity may contribute to the reduced effectiveness of the barley defence system against the non-adapted pathogen or promote/prevent *Bgt* pathogenicity factors. Many barley RILs had resistance factors to non-adapted mildew that prevented conidiation, barley lines showed a quantitative resistance at the seedling plant stage (Chantret *et al*. 2001). Parent Vada did not show any established colonies, and (nearly) all of penetration attempts, by *Bgt*, were stopped in association with papilla formation (Aghnoum & Niks 2010).

We observed that, for RILs with high establishment rate, there were some with positive conidiation, as well as others showing no conidiation. This suggests that on RILs on which *Bgt* establishes at high rates, the conidiation not necessarily is high. This is in agreement with studies by Aghnoum & Niks (2010) and Romero *et al*. (2018), that on DC and SC the rate of establishment by *Bgt* is similarly high, but the conidiation rate very different (Aghnoum & Niks

2010; Romero *et al*. 2018). We observed a score of *c*. 40 microcolonies/cm^2 on inoculated leaf samples (Table S2), similar to what was observed by Romero *et al*. (2018), 50 established microcolonies/cm^2 on SC and DC. Our results indicate about 10 % of the applied conidia resulted in established microcolonies compared to 50 % of Aghnoum & Niks (2010). We applied *Bgt* inoculum density of *c*.20 to 30 conidia/mm^2, and in the study of Aghnoum & Niks (2010), *c*. 50 conidia/mm^2 was applied (Aghnoum & Niks 2010). Almost all RILs had a lower score in establishment than parent DC and even the two most susceptible RILs in our current study were strongly resistant to establishment by *Bgt*. Our results confirmed that barley lines differ in their mean establishment and conidiation level by *Bgt*. Some of the RILs that did not have the Vada allele of the *Rbgqn1* QTL for resistance to establishment still had a very low establishment. Such low establishment is due to the remaining smaller effect QTLs for establishment (Fig. 2g; Fig. S2, S3). In our results barley RILs differed in the rate of conidiation, and the barley genetic factors to conidiation by *Bgt* are still unclear.

However, four barley RILs had too few established colonies, we could not reliably classify these barley RILs to either positive or negative. We have no clear explanation to the low establishment scores in these four RILs. Inoculation experiments were performed in summer, other factors such as low light intensity and low temperature might reduce *Bgt* development on barley RILs.

Recombinant inbred lines mapping populations are needed in the identification of resistance loci for the major diseases of barley. Our results demonstrated that resistance to non-adapted wheat mildew of barley was under polygenic control. The ordering of the barley RIL in a positive and negative conidiation group resulted in the identification of three chromosome regions. There was no chromosome region that was uniformly DC-derived for the positive and Vada-derived for the negative group (Fig. 2 a - g). This suggests that parent Vada encodes factor(s) that promotes conidiation, or parent SusBgtDC encodes a factor(s) that reduces/prevents conidiation. A basic compatibility exists between most formae speciales of *Blumeria graminis* and cereal species. In our results, *Bgt* development on barley REC RILs resulted in the complete life cycle, formation of conidia by the non-adapted mildew. Huckelhoven *et al*. (2001) reported that barley cv. Turkey allowed conidiation by *Bgt* (Hückelhoven *et al*. 2001). However, our results from the interaction of barley-*Bgt*, a small population (44 RILs) was probably insufficient to find all chromosome regions, segregating for conidiation by *Bgt*.

One or two major effect genomic regions can be found for disease resistance QTL analyses (Chantret *et al*. 2001). In our results, one genomic region had the greatest effect on conidiation by *Bgt* (1H) and the other two regions (6H) smaller effect. The marker significantly associated with conidiation found on 1H (8.6 cM) was within the same region (0 cM) as a minor QTL (*Rbgqn4*) associated with nonhost resistance in Vada x SusBgtDC population(Romero *et al*. 2018). Comparing the haplotype effect of the most significant marker 1H to the less significant marker (6HA), the mean conidiation scores were increased among the barley RILs having the positive allele (Vada) in both linkage groups (1H and 6H) compared to the barley RILs only having positive allele for either of two loci. This shows that 6H adds to the 1H effect. All nine REC RILs and a number of non-REC agreed at linkage 1H, this was not the same at 6H (6HA and 6HB), in which nearly all (eight) agreed and much of non-REC RILs did not agree (Fig. 2f). Three barley non-REC RILs that were positive for Vada allele at chromosome 6HA and 6HB did not show conidiation (Table S3), and their genotype for conidiation differed from the least convincing REC RIL DC-13 for conidiation on 1H (Table S3). These "deviating" RILs negative for conidiation had enough established colonies to be reliably sure about their negative conidiation status. The contrasting phenotype of these three RILs positive for Vada conidiation alleles at 6H, implies that the region found on chromosome 1H is most convincing for conidiation by the non-adapted mildew. Some disease resistance loci are effective to different strains of the same pathogen (Chantret *et al*. 2001). It is a challenge to find candidate genes in the 5.7 cM interval (1H) in which we presume the decisive factor is located, either in Vada (promoting factor) or in SusBgtDC (reducing factor). Hundreds of genes could be harboured on the region identified on chromosome 1H. We recommend fine-mapping to find the Vada or SusBgtDC candidate genes associated with conidiation by *Bgt*. For such fine-mapping we recommend the development of barley lines from a cross between the most convincing RILs positive and negative for conidiation. These barley lines are not selected only for contrasting conidiation, but establishment is high in both lines that allow reliable evaluation. Therefore, cross between RIL DC-23 x DC-105, for example, is needed to develop the F1 progeny. In heterozygous F1 plants we can determine the active factor for conidiation. We presume the genetic background is neutral for these three regions (1H and 6H), and molecular markers can be used. Phenotyping and genotyping of F2 barley seedlings for conidiation is needed. Such F2 plants if showing about high conidiation as RIL DC-23, the conidiation is dominant and that would suggest that Vada have a gene that promotes conidiation. Whereas no or poor conidiation as RIL DC-105 implies that no conidiation is dominant and parent SusBgtDC have a gene that reduces/prevents conidiation.

By phenotyping and genotyping F2 plants from DC-23 x DC-105 we can confirm the presence of the gene for conidiation. Recombinants around the three chromosome regions can be found and check which RILs still have the conidiation gene in the reduced region. Large enough in the number of F2 plants is required to determine the effect of conidiation loci. If locus 1H have high effect on conidiation than 6HA, suggests that these two loci are important and the gene is important for conidiation, and the combination of loci acts together. Whereas, similar or small difference in the effect of conidiation between loci show that the gene is not important for conidiation by non-adapted pathogen. Therefore, we can find out which allele is dominant: the Vada or the DC, that is important clue whether the functional gene promotes or prevents conidiation in the heterozygous plants.

We recommend phenotyping of the barley RILs, used in our study, to the adapted mildew resistance (*Blumeria graminis* f.sp. *hordei*) and determine the effectiveness of the chromosome regions found to *Bgh*. The barley chromosome regions found in the present study serve as useful genetic resource for Triticeae mildew resistance breeding programmes.

5. References

Aghnoum, R., & Niks, R. E. (2010). Specificity and levels of nonhost resistance to nonadapted *Blumeria graminis* forms in barley. *New Phytologist, 185*(1), 275-284. doi:doi:10.1111/j.1469-8137.2009.03039.x

Aghnoum, R., & Niks, R. E. (2011). Transgressive segregation for very low and high levels of basal resistance to powdery mildew in barley. *Journal of Plant Physiology, 168*(1), 45-50. doi:https://doi.org/10.1016/j.jplph.2010.09.005

Atienza, S. G., Jafary, H., & Niks, R. E. (2004). Accumulation of genes for susceptibility to rust fungi for which barley is nearly a nonhost results in two barley lines with extreme multiple susceptibility. *Planta, 220*(1), 71-79. doi:10.1007/s00425-004-1319-1

Boller, T., & Felix, G. (2009). A Renaissance of Elicitors: Perception of Microbe-Associated Molecular Patterns and Danger Signals by Pattern-Recognition Receptors. *Annual Review of Plant Biology, 60*(1), 379-406. doi:10.1146/annurev.arplant.57.032905.105346

Chantret, N., Mingeot, D., Sourdille, P., Bernard, M., Jacquemin, J. M., & Doussinault, G. (2001). A major QTL for powdery mildew resistance is stable over time and at two development stages in winter wheat. *Theoretical and Applied Genetics, 103*(6), 962-971. doi:10.1007/s001220100645

Chowdhury, J., Schober, M. S., Shirley, N. J., Singh, R. R., Jacobs, A. K., Douchkov, D., . . . Little, A. (2016). Down-regulation of the glucan synthase-like 6 gene (*HvGsl6*) in barley leads to decreased callose accumulation and increased cell wall penetration by *Blumeria graminis* f. sp. *hordei*. *New Phytologist, 212*(2), 434-443. doi:doi:10.1111/nph.14086

Cowger, C., Miranda, L., Griffey, C., Hall, M., Murphy, J. P., Maxwell, J., & Sharma, I. (2012). Wheat powdery mildew. *Disease resistance in wheat. CABI, Oxfordshire*, 84-119.

Dean, R., Van Kan, J. A. l., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., . . . Foster, G. D. (2012). The Top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology, 13*(4), 414-430. doi:doi:10.1111/j.1364-3703.2011.00783.x

Delventhal, R., Rajaraman, J., Stefanato, F. L., Rehman, S., Aghnoum, R., McGrann, G. R. D., . . . Schaffrath, U. (2017). A comparative analysis of nonhost resistance across the two Triticeae crop species wheat and barley. *BMC Plant Biology, 17*(1), 232. doi:10.1186/s12870-017-1178-0

Dodds, P. N., & Rathjen, J. P. (2010). Plant immunity: towards an integrated view of plant–pathogen interactions. *Nature Reviews Genetics, 11*, 539. doi:10.1038/nrg2812

Eichmann, R., & Hückelhoven, R. (2008). Accommodation of powdery mildew fungi in intact plant cells. *Journal of Plant Physiology, 165*(1), 5-18. doi:https://doi.org/10.1016/j.jplph.2007.05.004

Ferreira, R. B., Monteiro, S., Freitas, R., Santos, C. N., Chen, Z., Batista, L. M., . . . Teixeira, A. R. (2007). The role of plant defence proteins in fungal pathogenesis. *Molecular Plant Pathology, 8*(5), 677-700. doi:doi:10.1111/j.1364-3703.2007.00419.x

Hückelhoven, R., Dechert, C., & Kogel, K.-H. (2001). Non-host resistance of barley is associated with a hydrogen peroxide burst at sites of attempted penetration by wheat powdery mildew fungus. *Molecular Plant Pathology, 2*(4), 199-205. doi:doi:10.1046/j.1464-6722.2001.00067.x

Jafary, H., Szabo, L. J., & Niks, R. E. (2006). Innate Nonhost Immunity in Barley to Different Heterologous Rust Fungi Is Controlled by Sets of Resistance Genes with Different and Overlapping Specificities. *Molecular Plant-Microbe Interactions, 19*(11), 1270-1279. doi:10.1094/MPMI-19-1270

Lee, H.-A., Lee, H.-Y., Seo, E., Lee, J., Kim, S.-B., Oh, S., . . . Choi, D. (2017). Current Understandings of Plant Nonhost Resistance. *Molecular Plant-Microbe Interactions, 30*(1), 5-15. doi:10.1094/MPMI-10-16-0213-CR

Lee, S., Whitaker, V. M., & Hutton, S. F. (2016). Mini Review: Potential Applications of Non-host Resistance for Crop Improvement. *Frontiers in Plant Science, 7*(997). doi:10.3389/fpls.2016.00997

Niks, R. E., & Marcel, T. C. (2009). Nonhost and basal resistance: how to explain specificity? *New Phytologist, 182*(4), 817-828. doi:doi:10.1111/j.1469-8137.2009.02849.x

Niks RE, Parlevliet JE, Lindhout P, Bai Y (2011). Breeding crops with resistance to diseases and pests. Wageningen Academic Publishers, pp 198

Rajaraman, J., Douchkov, D., Hensel, G., Stefanato, F. L., Gordon, A., Ereful, N., . . . Schweizer, P. (2016). An LRR/Malectin Receptor-Like Kinase Mediates Resistance to Non-adapted and Adapted Powdery Mildew Fungi in Barley and Wheat. *Frontiers in Plant Science, 7*(1836). doi:10.3389/fpls.2016.01836

Romero, C. C. T., Vermeulen, J. P., Vels, A., Himmelbach, A., Mascher, M., & Niks, R. E. (2018). Mapping resistance to powdery mildew in barley reveals a large-effect nonhost resistance QTL. *Theoretical and Applied Genetics, 131*(5), 1031-1045. doi:10.1007/s00122-018-3055-0

Rsaliyev, A., Pahratdinova, Z., & Rsaliyev, S. (2017). Characterizing the pathotype structure of barley powdery mildew and effectiveness of resistance genes to this pathogen in Kazakhstan. *BMC Plant Biology, 17*(1), 178. doi:10.1186/s12870-017-1130-3

Schweizer, P. (2007). Nonhost resistance of plants to powdery mildew—New opportunities to unravel the mystery. *Physiological and Molecular Plant Pathology, 70*(1), 3-7. doi:https://doi.org/10.1016/j.pmpp.2007.07.004

Shtaya, M. J. Y., Marcel, T. C., Sillero, J. C., Niks, R. E., & Rubiales, D. (2006). Identification of QTLs for powdery mildew and scald resistance in barley. *Euphytica, 151*(3), 421-429. doi:10.1007/s10681-006-9172-x

Troch, V., Audenaert, K., Wyand, R. A., Haesaert, G., Höfte, M., & Brown, J. K. M. (2014). Formae speciales of cereal powdery mildew: close or distant relatives? *Molecular Plant Pathology, 15*(3), 304-314. doi:doi:10.1111/mpp.12093

VSN International (2018) Genstat for Windows 19th Edition. Webpage: GeneStat.co.uk, Hemel Hempstead, UK

Wolf, G., & Fric, F. (1981). A rapid staining method for *Erysiphe graminis* f. sp. *hordei* in and on whole barley leaves with a protein-specific dye. *Phytopathology, 71*(6), 596-598.

Yogendra, K. N., & Karre, S. (2016). Plant Innate Immune Response: Qualitative and Quantitative Resistance AU - Kushalappa, Ajjamada C. *Critical Reviews in Plant Sciences, 35*(1), 38-55. doi:10.1080/07352689.2016.1148980

6. Appendix: Supplementary Tables and Figures

Table S1 Summary of establishment results for other barley RILs negative for conidiation

Line	Est Cat[1]	Total Est	[2]Est frequency	Conid (%)	Genotype([3]REC_[4]EST)
DC-85	2	71	2.7 fghij	0	SSV_SVS
DC-38	1	68	2.4 hijk	0	SVS_SSS
DC-40	2	66	2.1 hijk	0	SVS_SSS
DC-100	2	63	2.1 hijk	0	SSS_SVV
DC-56	2	61	2.0 hijkl	0	SSS_VSS
DC-29	2	54	1.8 hijklm	0	SSS_VSS
DC-112	2	58	1.7 ijklmn	0	SVV_VSS
DC-107	2	57	1.7 ijklmn	0	SSV_SVS
DC-07	2	55	1.6 ijklmn	0	SVS_VSS
DC-49	3	50	1.5 ijklmn	0	SVS_VVS
DC-80	2	43	1.5 ijklmn	0	SSS_VVS
DC-75	2	36	1.2 jklmno	0	SSS_VVS
DC-77	2	36	1.2 jklmno	0	SVV_VVV
DC-61	3	34	1.1 jklmno	0	SSS_VVS
DC-89	2	33	1.1 klmno	0	VVS_SVV
DC-55	2	33	1.1 klmno	0	VVS_VVV
DC-42	2	32	1.0 klmno	0	VSS_VVV
DC-36	2	31	1.0 klmno	0	VSV_VSV
DC-90	3	21	0.6 lmno	0	VVV_VSS
DC-48	3	24	0.6 mno	0	VVS_VSV

[1]Establishment category: 1 = > 20 established microcolonies in each rep (n=3), 2 = in 1 or 2 reps established microcolonies > 20, 3 = no rep > 20, but total over reps > 20, and 4 = total over reps < 20
V = Vada, S = SusBgtDC
[2]Establishment frequency
[3]REC: Reproductive colonies – parent allele at 1H, 6HA and 6HB
[4]EST: Minor effect QTL of 2H, 4H and 1H for establishment by *Blumeria graminis* f.sp. *tritici*
The establishment and conidiation results are based on the average of three inoculation experiments for 44 RILs from Vada x SusBgtDC. For establishment frequency, recombinant inbred lines sharing the same letter are not significantly different ($P < 0.05$).

Table S2 Summary of inoculation experiment for barley lines with too few established colonies. Leaf age (six-day-old) and temperature (22/20°C) combination for establishment and conidiation by *Blumeria graminis* f.sp. *tritici.*

Line	SC	DC	*DC-17	*DC-20	* DC-73	*DC-109
Microcolonies/cm^2	40	40	0.57	0.45	0.40	0.50

Total number of barley lines analysed (six). *Four lines that had too few established colonies and SC, DC. Establishment scores are based on the average of two inoculation experiments for four barley lines.

Table S3 A subset of barley recombinant inbred lines contrasting for conidiation by *Blumeria graminis* f.sp.*tritici.* Three RILs negative for conidiation but positive for Vada allele at linkage 6H

RIL	[1]1H	[1]6HA	[1]6HB	[2]Conidiation
DC-13	V	V	V	Positive
DC-47	S	V	V	Negative
DC-104	S	V	V	Negative
DC-27	S	V	V	Negative

V = Vada, S = SusBgtDC
[1]Three chromosome regions (1H, 6HA and 6HA)
[2]For each RILs the conidiation type (positive or negative)

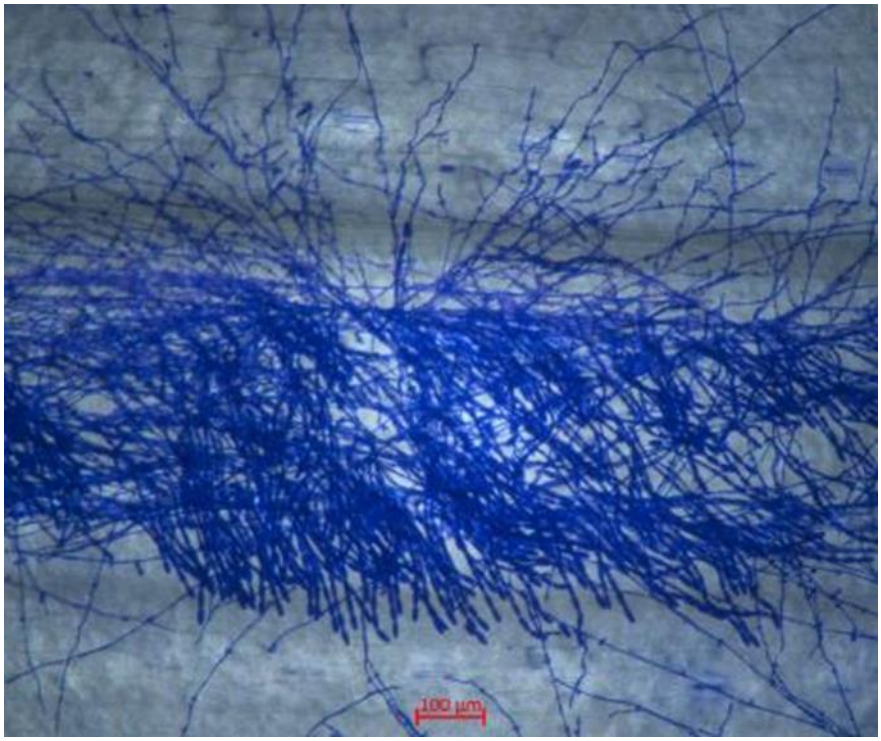


Fig. S1 Interaction between barley-*Blumeria graminis* f.sp. *tritici*. Merged *Bgt* colonies, four days after inoculation on barley at six-day-old and incubation temperature (22/20°C).

Marker	Chromosom	position (cM)	Vada	SusBgtDC	DC23	DC22	DC115	DC111	DC70	DC87	DC44	DC02	DC13	DC105	DC81	DC102	DC84	DC106	DC54	DC78	DC74	DC47	DC104	DC27	DC85	DC38
DC-C4_SNP8	4H	21.8	0	2	2	2	0	2	2	0	0	0	0	1	2	2	0	0	2	2	2	2	2	2	2	0
DC-C4_SNP9	4H	22.3	0	2	2	2	0	2	2	0	0	0	0	1	2	2	0	0	0	2	2	2	2	2	2	0
DC-C4_SNP10	4H	24.7	0	2	0	2	0	2	2	0	0	0	0	0	2	2	0	0	0	2	2	2	0	2	2	0
DC-C4_SNP11	4H	27.4	0	2	0	2	0	2	2	0	0	0	0	0	2	2	0	0	0	2	2	2	2	2	2	0
DC-C4_SNP12	4H	28.3	0	2	0	2	0	2	2	0	0	0	0	0	2	2	0	0	0	2	2	2	2	2	2	0
DC-C4_SNP13	4H	31.7	0	2	0	2	0	2	0	0	0	0	0	0	2	2	0	0	0	0	2	2	2	2	2	0
DC-C4_SNP14	4H	35.1	0	2	0	2	0	2	0	0	2	0	0	0	2	2	0	0	0	0	2	2	2	2	0	0
DC-C4_SNP15	4H	38.9	0	2	2	2	0	2	0	2	2	0	0	0	2	1	0	0	0	0	2	2	2	2	0	0
DC-C4_SNP16	4H	40.8	0	2	2	2	0	2	2	2	2	2	0	0	2	1	2	2	0	0	2	2	2	2	0	0
DC-C4_SNP17	4H	45.8	0	2	2	2	0	0	2	2	2	2	0	0	2	1	2	2	0	0	2	2	2	2	0	2
DC-C4_SNP18	4H	49.7	0	2	2	2	0	0	2	2	2	2	0	0	2	1	0	0	0	0	2	2	2	2	0	2
DC-C4_SNP19	4H	52.4	0	2	2	2	0	0	2	2	2	2	0	0	2	1	0	0	0	0	2	2	2	2	0	2
DC-C4_SNP20	4H	54.2	0	2	2	2	0	0	2	2	2	2	0	0	2	0	0	0	0	0	2	2	2	2	0	2
DC-C4_SNP21	**4H**	**57.4**	**0**	**2**	**2**	**2**	**0**	**0**	**2**	**2**	**2**	**2**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**2**	**2**	**2**	**0**	**2**
DC-C4_SNP22	4H	61.9	0	2	2	2	0	0	2	2	2	2	0	1	0	1	0	0	0	0	0	2	2	2	0	0
DC-C4_SNP23	4H	64.9	0	2	2	2	0	0	2	2	2	2	0	0	0	0	2	2	0	0	0	2	2	2	0	2
DC-C4_SNP24	4H	67.2	0	2	0	2	2	0	2	2	2	2	0	1	0	0	2	2	0	0	0	2	2	2	0	2
DC-C4_SNP25	4H	70.6	0	2	2	2	2	0	2	2	2	2	0	1	2	2	2	2	0	0	0	2	2	2	0	2
DC-C4_SNP26	4H	73.0	0	2	0	2	2	0	2	2	2	2	0	1	2	2	0	0	0	0	0	2	2	2	0	2
DC-C4_SNP27	4H	75.3	0	2	0	2	2	0	2	2	2	2	0	2	2	2	0	0	0	0	0	2	2	2	0	2
DC-C4_SNP28	4H	79.6	0	2	0	2	2	0	2	0	0	2	0	2	2	2	0	0	0	0	0	2	2	2	0	2
DC-C4_SNP29	4H	82.5	0	2	0	2	2	0	2	0	0	2	2	2	2	2	0	0	0	0	0	0	2	2	0	2
DC-C4_SNP30	4H	85.7	0	2	2	2	2	0	2	0	0	2	2	2	2	2	0	0	0	0	0	2	2	2	0	0
DC-C4_SNP31	4H	87.1	0	2	2	2	2	0	2	0	0	2	2	2	2	2	0	0	0	0	0	2	2	2	0	0
DC-C4_SNP32	4H	90.7	0	2	2	0	2	0	0	0	0	2	2	2	2	2	0	0	0	0	0	2	2	2	0	0
DC-C4_SNP33	4H	90.7	0	2	2	0	2	0	0	0	0	2	2	2	2	2	0	0	0	0	0	2	2	2	0	0

4H

Rbgqn3

Fig. S2 Minor QTL for establishment at 4H (*Rbgqn3*). Red = Vada and Green = SusBgtDC

Marker	Chromosom	position (cM)	Vada	SusBgtDC	DC23	DC22	DC115	DC111	DC70	DC87	DC44	DC02	DC13	DC105	DC81	DC102	DC84	DC106	DC54	DC78	DC74	DC47	DC104	DC27	DC85	DC38
DC-C1_SNP1	**1H**	**0.0**	**0**	**2**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
DC-C1_SNP2	1H	2.9	0	2	0	0	0	0	0	0	0	0	2	0	2	2	2	2	2	2	2	2	2	2	2	2
DC-C1_SNP3	1H	5.3	0	2	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2
DC-C1_SNP4	1H	8.6	0	2	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2
DC-C1_SNP5	1H	11.0	0	2	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2
DC-C1_SNP6	1H	14.9	0	2	0	0	0	0	0	0	0	0	2	1	2	2	2	2	2	2	2	2	2	2	2	2
DC-C1_SNP7	1H	17.2	0	2	0	0	0	0	0	0	2	2	2	1	0	0	2	2	2	2	2	2	2	2	2	2
DC-C1_SNP8	1H	19.6	0	2	0	0	2	0	0	0	2	2	2	1	0	0	2	2	2	2	2	2	2	2	2	2
DC-C1_SNP9	1H	22.9	0	2	0	0	2	0	0	0	2	2	2	1	0	0	2	2	2	2	2	2	2	2	2	2
DC-C1_SNP10	1H	25.3	0	2	0	0	2	0	0	0	2	2	2	1	0	0	2	2	2	2	2	2	2	0	2	2
DC-C1_SNP11	1H	28.6	0	2	0	0	2	0	0	0	2	2	0	1	0	0	2	2	2	2	2	2	2	0	2	2
DC-C1_SNP12	1H	31.8	0	2	0	0	2	0	0	0	2	2	0	1	0	0	2	2	2	2	2	2	2	0	2	2
DC-C1_SNP13	1H	33.6	0	2	0	0	2	0	0	0	2	2	0	2	0	0	2	2	2	2	2	2	2	0	2	2
DC-C1_SNP14	1H	37.4	0	2	0	0	2	0	0	0	2	2	1	1	0	0	2	2	2	2	0	2	2	0	2	2
DC-C1_SNP15	1H	40.2	0	2	0	0	2	0	0	0	2	2	2	1	0	0	2	2	2	2	0	2	2	2	0	2
DC-C1_SNP16	1H	44.1	0	2	2	0	2	0	0	0	2	2	2	1	0	0	2	2	2	2	0	2	2	2	0	0
DC-C1_SNP17	1H	47.7	0	2	2	0	2	0	0	0	2	2	2	1	0	0	2	2	2	2	0	2	2	2	0	0
DC-C1_SNP18	1H	50.1	0	2	2	2	2	0	0	0	2	2	2	2	0	0	2	2	0	2	0	2	2	2	0	0
DC-C1_SNP19	1H	53.3	0	2	2	2	2	0	0	0	2	2	2	2	0	0	2	2	0	2	0	2	2	2	0	0
DC-C1_SNP20	1H	56.7	0	2	2	2	2	0	0	0	2	2	2	2	0	0	2	2	0	2	0	0	2	2	0	0
DC-C1_SNP21	1H	59.9	0	2	2	2	2	0	0	0	2	2	2	2	2	2	2	2	0	0	0	0	2	2	0	0
DC-C1_SNP22	1H	62.2	0	2	2	2	2	0	0	0	2	2	2	2	2	2	2	2	0	0	0	0	2	2	0	0
DC-C1_SNP23	1H	64.0	0	2	2	2	2	0	0	2	2	2	2	2	2	2	2	2	0	0	0	0	2	2	0	0
DC-C1_SNP24	1H	67.4	0	2	2	2	2	0	0	2	2	2	2	2	2	2	2	2	0	0	0	0	2	2	0	0
DC-C1_SNP25	1H	69.8	0	2	2	2	0	0	0	2	2	2	2	2	2	2	2	2	0	0	0	0	2	2	0	0
DC-C1_SNP26	1H	72.6	0	2	2	2	0	0	0	2	2	2	2	2	2	2	2	2	0	2	0	0	2	2	0	0

1H

Rbgqn4

Fig. S3 Minor QTL for establishment at 1H (*Rbgqn4*). Red = Vada and Green = SusBgtDC