Mapping *Bremia Lactucae* non-host resistance from *Lactuca saligna*

MSc-thesis

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Preface

This thesis is part of my MSc study Plant Sciences. I applied on an available project of Marieke Jeuken because I wanted to gain some more experience in research about quantitative genetics. I started with this thesis in June 2012 and did most of the activities for this thesis including collecting all data (genotyping + phenotyping), and data analysing until February 2013. From March 2013 until March 2014 I was busy with other activities than this thesis. I participated in the academic consultancy training and did a long internship. With some re-start difficulties I finished this report in May 2014.

I want to thank Marieke Jeuken for introducing me into the subject of resistance QTL analysis. I learned how to combine phenotypic and genotypic data to predict resistance QTLs. Furthermore, I want to thank Erik den Boer who explained me how to detect SNPs with high resolution melting curve analyses.

Abstract

In this thesis four *B. lactucae* non-host resistance QTLs from *L. saligna* were mapped. Two of them were mapped in a region which corresponds with *L. saligna* introgression regions associated with *B. lactucae* resistance of two different Backcross Inbred Lines. The two other QTLs had peaks on the same markers as found in earlier tested F_2- and BC- mapping populations. One of these QTLs was until now only presented in *L. saligna* CGN15705 and not in *L. saligna* CGN05271.

The mapping of *B. lactucae* non-host resistance was done in a backcross mapping population of 63 plants originating from a cross between *L. sativa* cv. Olof and *L. saligna* CGN15705, backcrossed in *L. sativa* cv. Olof. These plants were genotyped with a set of 79 EST-based SNP markers and phenotyped in three disease tests with two *Bremia lactucae* races (Bl:21 and Bl:24), performed on adult greenhouse plants of a backcross mapping population.

As extra thesis activities, morphological traits were mapped in the backcross mapping population, with significant QTLs found for early bolting, side shoots, dark green leafs, twisted leafs, and leaf tip shape. Progeny of the backcross population with *L. saligna* CGN05271 as parent was tested for epistatic effect between QTL loci, which was found before but not confirmed in this thesis. Also the F_1 between between *L. sativa* cv. Olof and a new *L. saligna* accession CGN05310 was tested in a disease test with young plants with two different *B. lactucae* race (Bl:21 and Bl:24) and not surprisingly, the F_1 plants were found to be resistant.

Keywords: *Bremia lactucae*; *Lactuca saligna*; *Latcuca sativa*; mapping; QTL; resistance

List of abbreviations

a	homozygote *L. sativa* cv. Olof genotype
ADTg	adult plant disease with greenhouse plants
b	homozygote *L. saligna genotype*
BC	backcross
BIL	Backcross Inbred Line
Bl	*Bremia lactucae (strain)*
C	chromosome
D	dark
dpi	days post inoculation
dps	days post sowing
FR	France *L. saligna* CGN05271
GEO	Georgian *L. saligna* CGN15705
h	hetereozygote genotype
HRM	high resolution melting
IM	Interval Mapping
ISL	infection severity level
ISR	Israelian *L. saligna* CGN05310
KW	Kruskal-Wallis test
L	light
L.	*Lactuca*
QTL	Qantitative Trait Locus
sat	*L. sativa*
SNP	single nucleotide polymorphism
u	unknown genotype
YDT	young plant disease test

1 Introduction

1.1 *Bremia lactucae* in *Lactuca* species

Bremia lactucae is a pathogen which infects the cultivated Lettuce (*Lactuca sativa*) causing the disease lettuce downy mildew (see textbox 1: *Bremia lactucae*). Yellowing of the leafs with necrotic spots on the leafs are the symptoms of lettuce downy mildew (Verhoef 1960) making the lettuce unsalable. Resistance against *B. lactucae* is therefore an important trait for lettuce breeders.

Until now most *B. lactucae* resistance in cultivated lettuce is hypotized to be based on a gene-for-gene interaction between R-genes in the lettuce and avirulence genes from *B. lactucae.* This resistance mechanism is race-specific, since each *B. lactucae* race has a different set of avirulence genes and each R-gene is only available to give resistance to the *B. lactucae* races with the corresponding avirulence gene. This monogenic R resistance is often associated with the Hypersensitive Response (HR), which is a strong resistance mechanism (Crute and Johnson 1976). These R-genes in lettuce are named *Dm* (Downy mildew) genes for *B. lactucae* resistance. At least 24 *Dm* genes are known according to the International *Bremia* Evaluation Board (IBEB, 2010).

Dm-genes are found on three gene clusters on chromosome 1, 2 and 4, only one Dm-gene is found lonely on chromosome 3 (McHale et al. 2009). *R*-genes are known to contribute to resistance mechanisms which are non-durable if simply introgressed into a susceptible background (Pink 2002). A single base mutation in the corresponding avirulence gene of the pathogen can already be enough to overcome the resistance. *B. lactucae* has a mixed reproduction system (sexual and asexual), therefore a high evolutionary potential (McDonald and Linde 2002). Mutations and sexual recombination give raise to new genotypes. The newly arisen genotypes with a high fit can than establish very fast on lettuce fields by asexual reproduction and the help of spores dispersion by wind and rain. At least 29 European *B. lactucae* races are currently described (IBEB, 2010).

The current breeding for *B. lactucae* resistance in lettuce is predominately focussed on monogenic qualitative resistance caused by interaction between a plant R-gene and a pathogen Avr-gene. A more durable resistance would be good for a more stable production of lettuce. A possible source of a more durable resistance could be found in *Lactuca saligna* (see textbox 2). *L. saligna* is regarded as a non-host of *B. lactucae* and although not in the same gene pool with *L. sativa*, it is with some effort crossable with cultivated lettuce.

Textbox 1: *Bremia lactucae*

Bremia lactucae is the pathogen causing lettuce downy mildew. A disease in lettuce for which in some regions, growers use 60% or more of their fungicides to fight this disease (iMetos 2012).
The symptoms of the disease are the presences of light green to yellow leaf spots which are delineated by veins, especially in older leafs with thicker veins (Verhoef 1960) . At the lower side of the leaf these spots are often covered by white tufts consisting of conidiophores with conidia. The conidiophores can also be on the upper side of the leaf when lettuce plants are growing close together. Besides these symptoms, necrotic spots can arise in the centre of the yellowing leaf spots.

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B. lactucae is an obligate parasite belonging to the group op oomycetes, belonging to the family Peronosporaceae. It is known to infect over 200 species in 40 different genera within the Compositae (Crute and Norwood 1981; Lebeda et al. 2002; Lebeda et al. 2008). *L. sativa* is the only economic important host infected by *B. lactucae.* Lettuce is one of the 10 most valuable crops in the United States with an annual value of over 2.7 billion dollars, and similar value in Europe(USDA NASS 2008). Fungicides could be used to control *B. lactucae*, but tolerant isolates against these chemical protectants are reported (Schettini et al. 1991)(Schettini et al. 1991).

B. lactucae has both a sexual as an asexual lifecycle (Figure T1). The asexual lifecycle takes 1 to 3 weeks and the sexual cycle can take several weeks to many years (Michelmore et al. 2009). The asexual lifecycle consist of the formation of asexual spores called conidia in the conidiophore (Padgett-Johnson and Leammlen). The wind spreads the conidia. The conidium will geminate when it falls on its host leaf and enters the lettuce leaf via direct penetration of epidermal cells or via stomata. Hyphae develop and penetrate other leaf cells as source of nutrition. Under the right climate conditions and after a certain amount of mycelium formation in the leaf tissue, sporulation occurs and new conidiophores bearing conidia develop en this cycle repeats. Besides conidia, some isolates produce asexual zoospores homothallic, which develops from a conidia forming into a sporangium in between (Michelmore and Sansome 1982). Zoospores are also the final product after sexual reproduction. An antheridium and an oognonium are able to form an sexual oospore. The anteridium and oogonium have to be from another mating type to form oospores. These oospores contain the valuable meiotic recombinations, the origin of new *B. lactucae* isolates (Michelmore and Ingram 1980). The oospore subsequently germinates into a sporangium forming the asexual oospores.

Temperature, humidity and wind affects the conidia sporulation of *B. lactucae* (Su et al. 2004). The optimum temperature range for sporulation is between 10 and 20°C, for isolates from colder climates it seems to be more toward 10°C, for isolates from warmer climates it is more towards 20°C. The relative humidity should be above 90%, lower relative humidity gives no sporulation and wind speeds above 0.5 m/s also inhibits sporulation completely.

Textbox 1: *Bremia lactucae* – continued

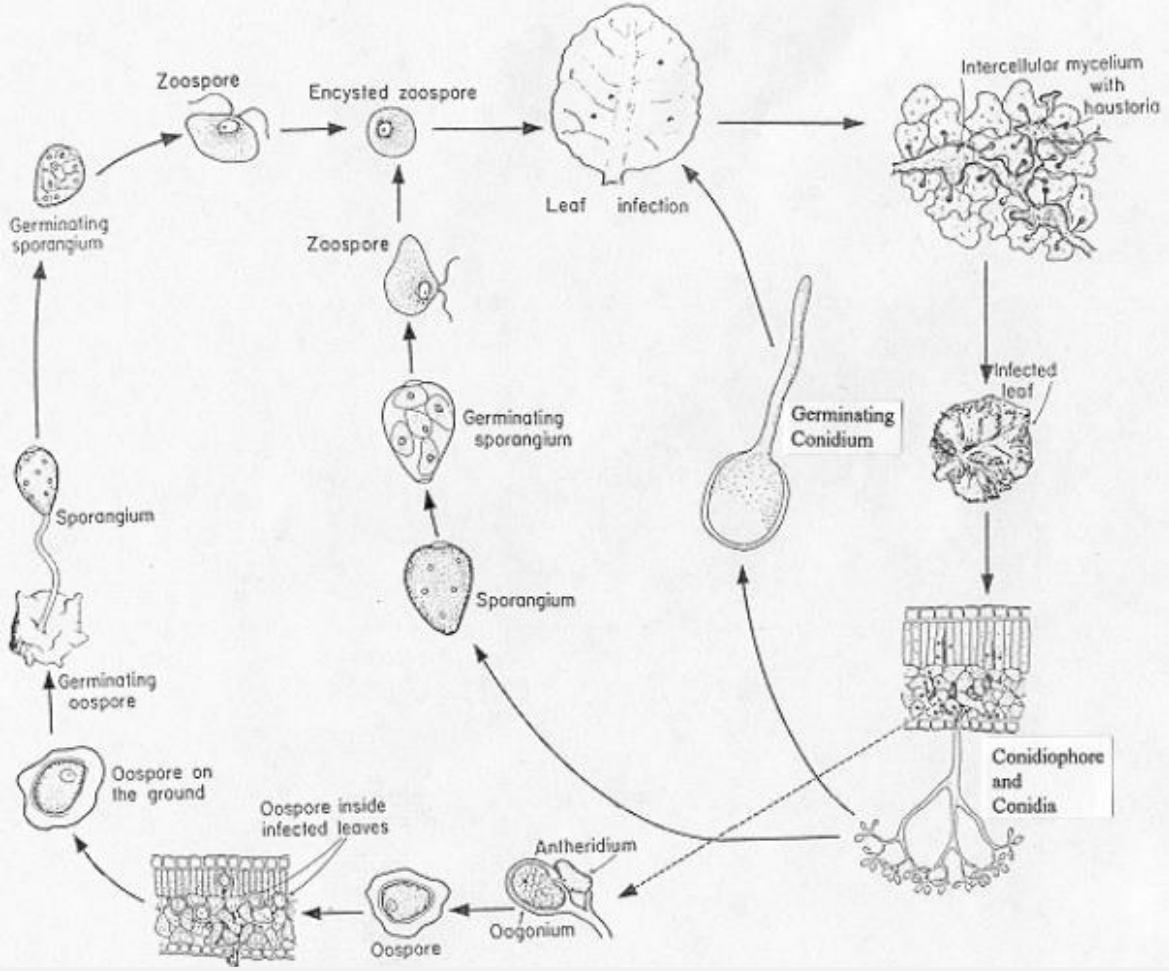

Figure T1 Disease cycle of lettuce downy mildew caused by *Bremia lactucae* (Padgett-Johnson and Laemmlen). The asexual lifecycle mainly exist of the formation of conidia in conidiophore. In some *B. lactuca*e isolates an secondary asexual life cycle homothalic zoospores develop from a sporangium, so this pathway is an secondary asexual lifecycle. The sexual lifecycle also consists of the formation from zoospores from sporangia after an antherdium and oogonium from isolates with different mating types formed an oospore.

Textbox 2: *B. lactucae* resistance in *L. sativa* gene pool

Lactuca sativa is the cultivated lettuce, member of the genus *Lactuca* and of the Asteraceae family . With an phylogenic study on basis of DNA sequence the primary and secondary gene pool were determined (Koopman et al. 1998). This study grouped the species *L. serriola, L. dregeana, L. altaica, and L. aculeata* as the primary gene pool of *L. sativa*. In the second gene pool *L. virosa* and *L. saligna* were grouped. These latter species are crossable with *L. sativa*, but with less viable descendants per cross than with the plants from the primary gene pool.

In a search for new more durable resistance sources multiple accessions were tested in four *Lactuca* species: L. *saligna* (n=55 accessions), *L. virosa* (n=67), *L. serriola* (n=126) and *L. sativa* (n=187), each accession is tested with at least 13 different *B. lactucae* isolates. The results of these diseases tests can be found in Figure T2 (unpublished data MJW Jeuken). As can be seen, there is large variance in Infection severity levels within *L. sativa*, *L. serriola* and *L. virosa*, but not in *L. saligna*. All tested accessions of *L. saligna* gave low infection severity levels and are highly to absolute resistant. Although not all existing accessions of *L. saligna* were tested, *L. saligna* is considered as a non-host of *B. lactucae*. This non-host status of *L. saligna* was also concluded in an earlier study (Bonnier et al. 1992).

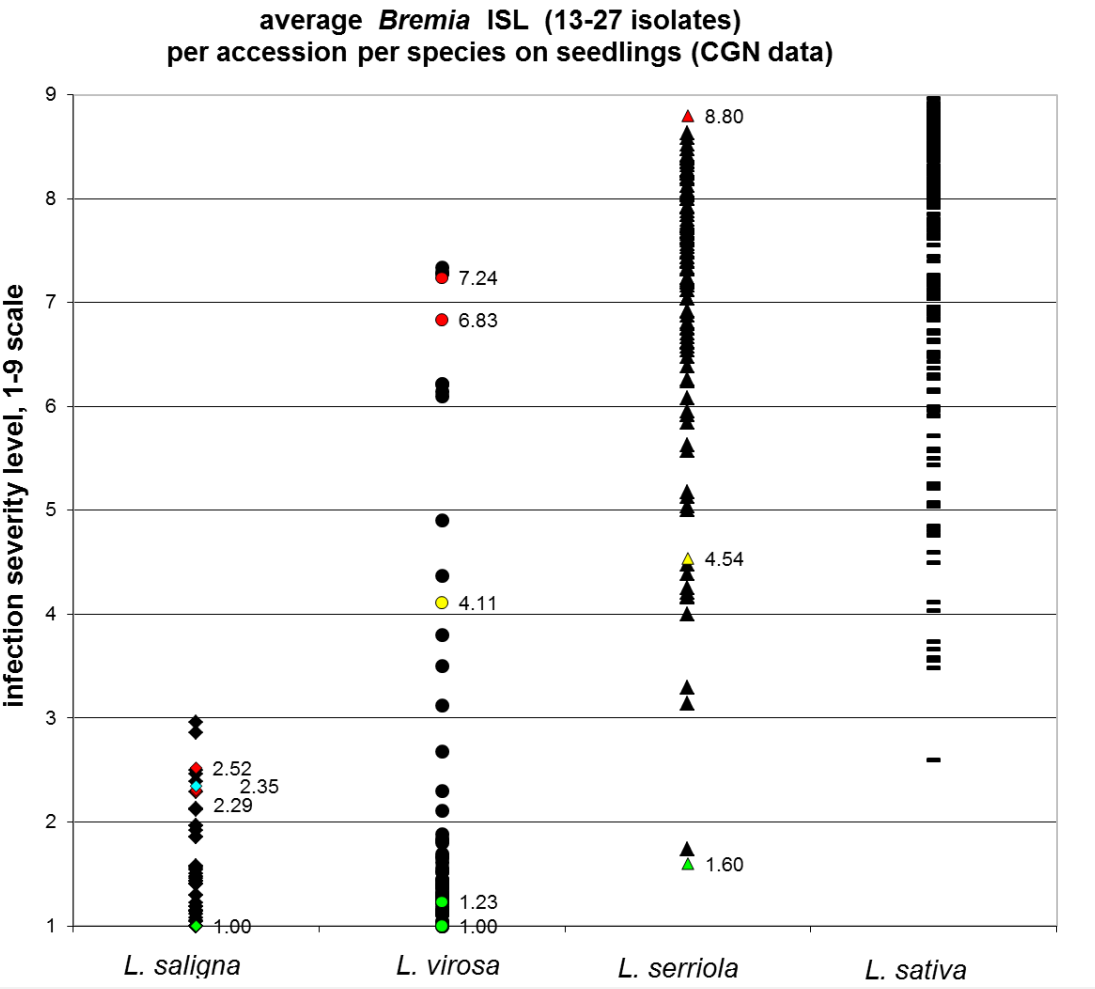

Figure T2 The mean Infection Severity Level on seedlings level per accession within the species *L. saligna* (n=55 accesions), *L. virosa* (n=67), *L. serriola* (n=126) and L. sativa (n=187). Each value represents an accessions tested with

1.2 Understanding of the *B. lactucae* non-host resistance

The non-host resistance against *B. lactucae* in *L. saligna* is promising for breeding for more durable *B. lactucae* resistance in lettuce cultivars. Understanding of the *B. lactucae* non-host resistance could speed up the breeding process for more durable resistance. For example by the use of markers associated with the non-host resistance responsible genetic elements in marker assisted selection. For complete insight of the mechanism of the non-host resistance from *L. saligna* it would be necessary to know all metabolites and pathways involved in this mechanism. Before it will be possible to reveal these participants of the resistance mechanism, the responsible proteins in these pathways should be discovered. These proteins are coded by genetic elements. Before we can discover the genetic codes of these genetic elements they should be localized.

On this moment the focus is on the mapping of the genetic elements, their effect and the interaction effects. Evidence that the *B. lactucae* non-host resistance is a quantitative resistance, involving Quantitative trait loci (QTLs), was found in the segregation pattern of infection severity levels (ISLs) between the progeny of several hybrid crossings between *L. saligna* and *L. sativa* (see Figure 2)(den Boer et al. 2014). A continuous ISL segregation pattern was found within a backcross population arisen from a F_1 (*L. saligna* from France x *L. sativa* cv. Olof) backcrossed with the *L. sativa* cv. Olof (BC1_FR_sat in Figure 2) and in a F_2-pulations, originating from a hybrid cros between a *L. saligna* from France and *L. sativa* cv. Olof(Jeuken and Lindhout 2002). An overview of the made crosses and the number of phenotyped plants can be seen in Figure 1. All phenoytped progeny populations started with an initial hybrid crosses between the highly susceptible *L. sativa* cv. Olof as father and three non-host L. saligna accessions CGN15705, CGN05271, 275-5as mothers collected from respectively Georgia (GEO), mainland France (FR) and the island Corsica (CO). Backcrosses were made and F2 populations were made. For the F2, a selection was made. Only vital plants with extreme resistance phenotypes, highly resistant and the most susceptible, were selected (F_2_selected).

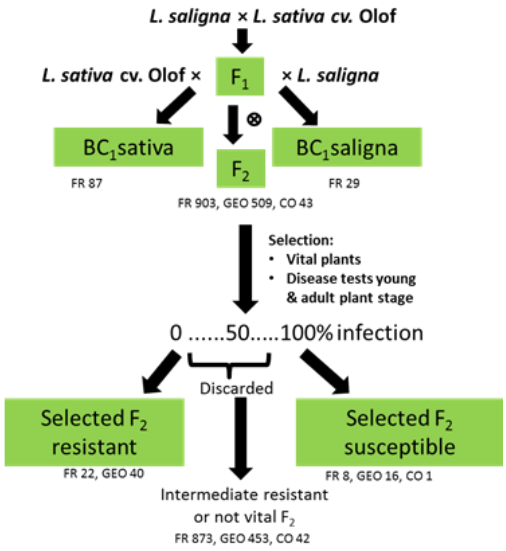

Figure 1. Crossing scheme for the genetic dissection of the downy mildew resistance in three *L. saligna* accessions, CGN05271 (FR), CGN15705 (GEO) and 275-5 (CO). Green boxes indicate that the population is phenotyped for resistance. The number of phenotyped plants are shown under the green boxes per *L. saligna* accession cross. This figure is adjusted from den Boer et al. 2014.

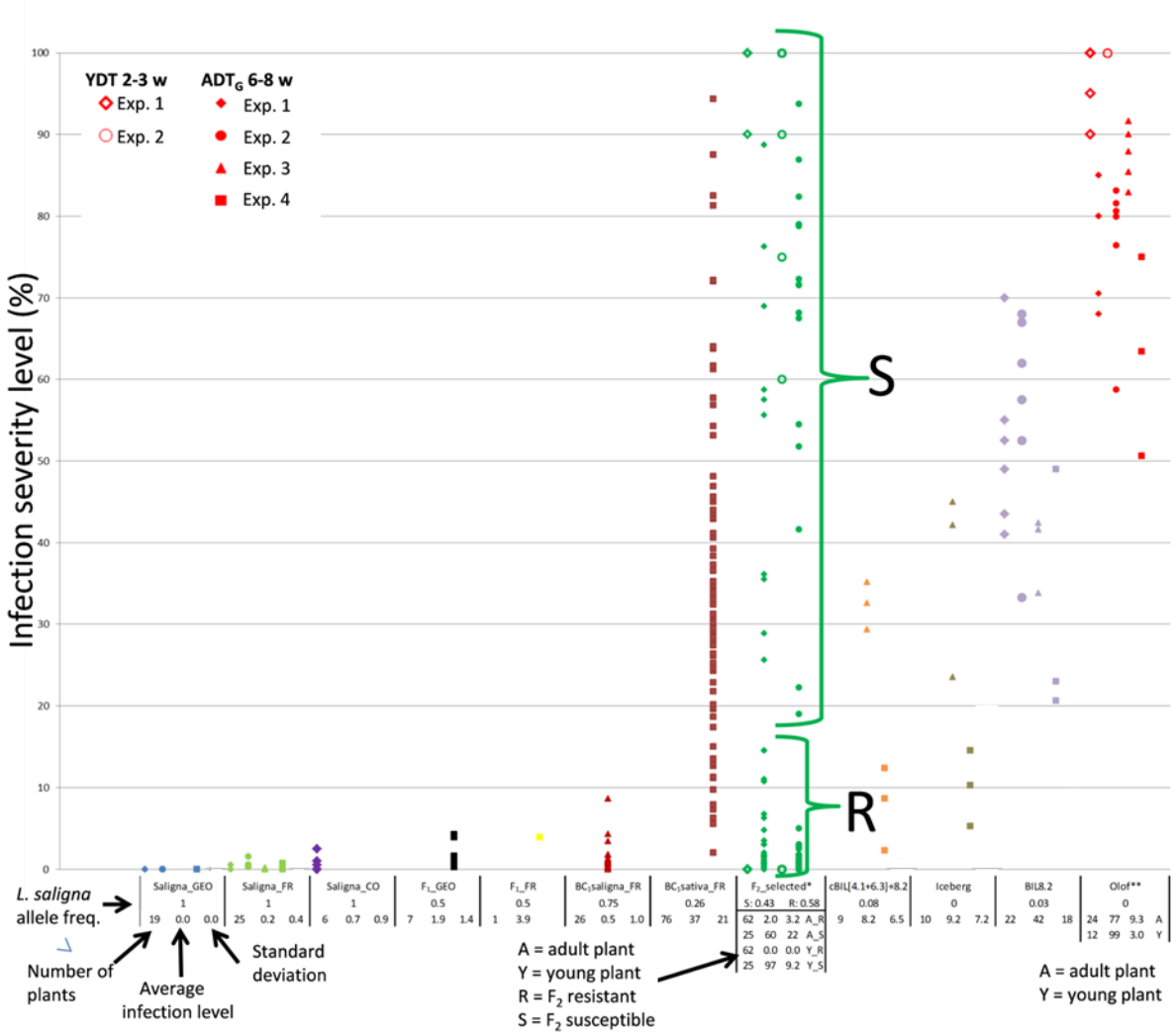

Figure 2. Infection severity plots from control lines and populations in six experiments (two YDT and four ADT$_G$). Plants with a hybrid necrosis phenotype are not included. For the F_2 population R and S mean highly resistant and susceptible respectively. Only the selected and genotyped F_2 plants are shown from all three *L. saligna* × *L. sativa* populations, derived from the following *L. saligna* accessions as mother; CGN05271 (FR), CGN15705 (GEO) and 275-5 (CO) crossed with *L. sativa* cv. Olof. Each line / population is visualized by a different color. Per experiment the control lines and the population(s) are visualized by a different symbol. This figure is adjusted from den Boer et al. 2014.

In one of the earliest attempts to localize these genetic elements (assumed to be QTLs) a F_2 mapping population (n=126) (not mentioned in figure 1 and 2) from a cross between the resistant *L. saligna* CGN5271 (FR) x susceptible *L. sativa* cv. Olof was used. Disease tests were done with *B. lactucae* races (Bl:) 14 and 16. Four possible QTLs were found, on top of chromosome (C)1 (nearly significant QTL for Bl:16), the bottom of C7 (significant for Bl:14), the top of C9 (significant for Bl:16) and just behind the top of C9 (barely signficant for Bl:14). (Jeuken and Lindhout 2002). The assumed QTL on the top of C9, was actually proven to be *Rin4*, coding for an assumed guard protein which causes an necrotic autoimmune response interacting with a assumed NBS-like R-gene on the top of C6(Jeuken, Zhang et al. 2009). This is an auto-immune response triggered resistance.

At least 15 other QTLs were found in screening a set of 29 backcross inbred lines (BILs), each line conferring an introgression of *L. saligna* in *L. sativa*. Combining these QTLs in combiBILs gave

complete and almost complete resistance in a tripleBIL and a quatroBIL. This was not found for all comiBILs, in some cases combining introgressions gave the same resistance level or even lower resistance levels. This indicates that there is a variety of epistatic interactions between the QTLs. (Brown et al. 2004; Jeuken et al. 2008; Zhang et al. 2009). Several attempts to map the QTLs in resistance contributing introgressions gave as result that more than one QTL is responsible in each introgression (personal communication Boer).

More recent attempts for mapping the responsible resistance QTLs were done with three F_2-populations and a BC_1-population(den Boer et al. 2014). These F_2 populations were obtained by crosses between *L. sativa* cv. Olof as father and *L. saligna* accessions as mothers from Georgia (GEO), France (FR) and Corsica (CO) as mentioned earlier and presented in Figure 1. Phenotype results from disease tests with Bl:21 were shown in Figure 2. The phenotypic data was combined with genotypic data in mapping software and analysed with the Kruskal-Wallis test. For the F_2-populations only the extreme phenotypes (very high and very low ISLs) were selected to genotype. For the F_2_CO there were no plants with very low ISL and only one plant with very high ISL, therefore this population is not used for mapping.

The outcome of mapping the *B. lactucae* resistance in the F_2_FR, the F_2_GEO and the BC_1_sat_FR mapping populations is found in Figure 4. For the selected F_2_FR significant QTLs were found for the middle of C6 and a nearly significant QTL at the end of C1. For the selected F_2_GEO significant QTLs were found at the end of C1, the middle of C6, the end of C7 and the end of C9. For the BC_1_FR significant QTLs were found at the middle of C6, the middle of C7 and the begin of C9. It appears that the QTLs on the end of C9 and the end of C1 are specific for the accession from Georgia. The significance and the effect of the QTL at the end of C9 is so large that it could be that this QTL is a R-gene instead of a QTL.

Significant epistatic interaction between the QTL on the middle of C6 with the QTLs on the bottom of C1 and at the middle of C7 were detected in the F_2 populations. Significant increased *B. lactucae* resistance (equals lowered ISLs) was only reached when there was a *L. saligna* allele at the middle of C6 in combination with a *L. saligna* allele at the bottom of C1 or at the middle of C7, see Figure 3.

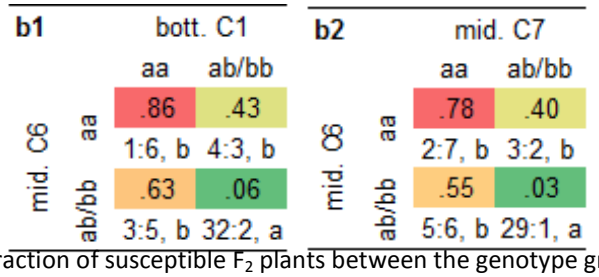

Figure 3. Comparison of fraction of susceptible F_2 plants between the genotype groups of the resistance loci involved in the epistatic interaction, mid C6, bott C1 and mid. C7. The resistance conferred by *L.* saligna alleles, was mainly dominant, therefore only the genotype groups with absence (homozygous *L. sativa*, "aa") and presenence (heterozygous "ab" or homozygous *L. saligna* "bb") of *L. saligna* alleses are shown. Gradual color scale is used to visualize differences in faction of susceptible plants (susceptible / all plants) (green: higher proportion of resistant, to red: higher proportion of susceptible plants in the genotype class). Under each colored box the fraction of susceptible plants is indicated. The letter between the colored box indicates whether the fraction of susceptible plants between the genotypes within subtable b1 or b2 are significant different. This is the case if the letter is different (Fisher's Exact test, α=0.05. This figure is adjusted from den Boer et al. 2014.

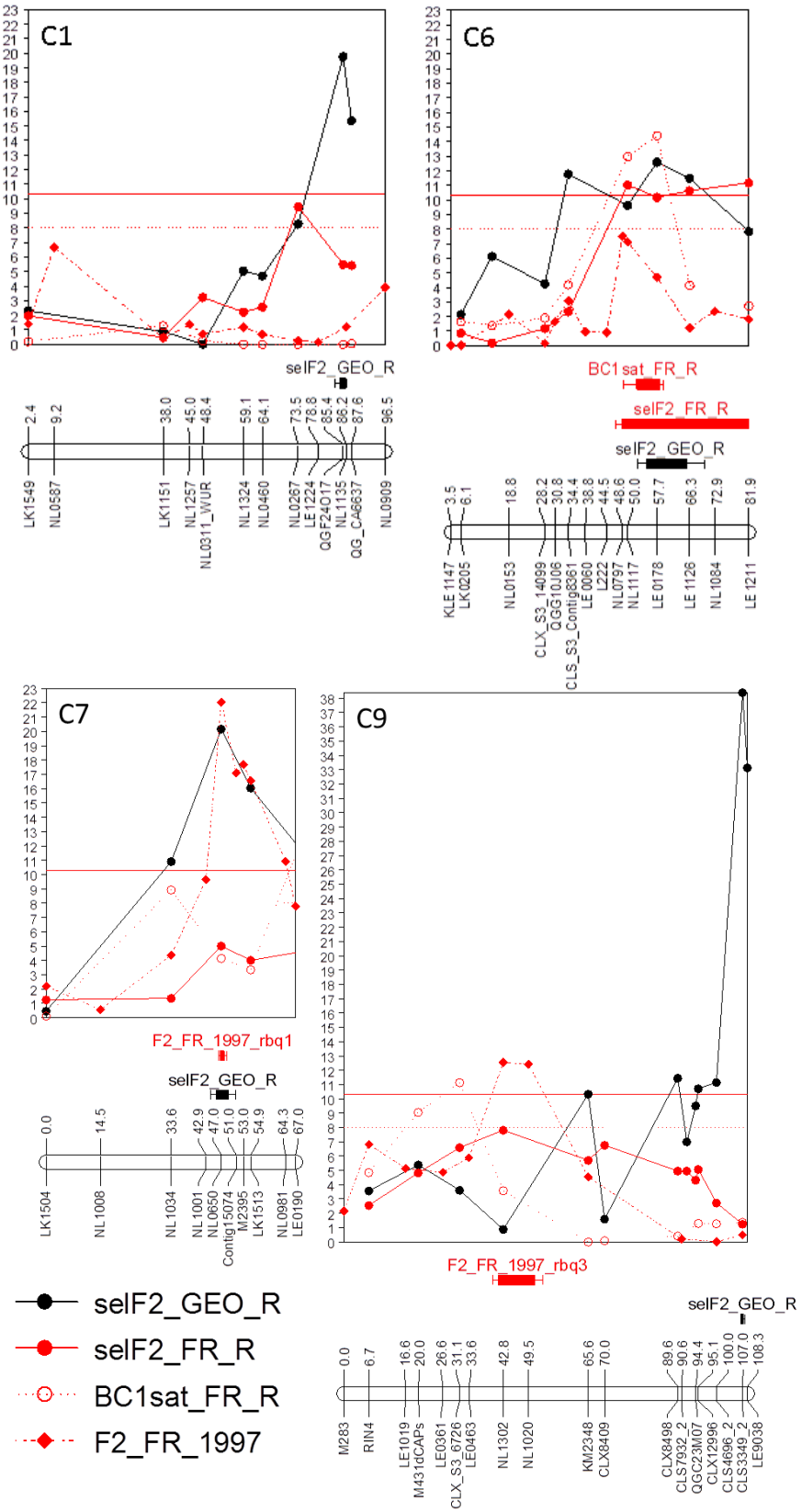

Figure 4. Resistance loci at Chromosome 1, 6, 7 and 9 detected in four populations. Kruskal-Wallis test results (K values) are plotted for linkage groups in which significant peak values were found, with an α=0.005 threshold; horizontal red lines, solid for F_2 and dotted for BC_1sativa. Lines present K values for populations derived from a *L. saligna* CGN05271 in red and from CGN15705 (GEO) in black. (Figure and data by Erik den Boer, unpublished results)

1.3 Thesis Goal

Mapping *B. lactucae* resistance in different population types and from different *L. saligna* accessions gave two resistance loci that were similar between two L. saligna accessions and two loci that were lineage specific (GEO). By mapping *B. lactucae* resistance in an additional BC_1-population (n= +-72 seeds) from the Georgian *L. saligna* CGN15705(BC_1_sat_GEO), more data about locations of *Bremia* resistance QTLs will be collected. Hypotized is that a new data will confirm the four earlier detected loci. Also interesting is the peak at the bottom of C9, which was until now only found in a mapping population with a *L saligna* parent from Georgia. So expected is to see this peak also in the BC_1_sat_GEO population. In general could be said that this extra data could give a better understanding of the non-host resistance against *Bremia* from *L. saligna.*

Besides the population mentioned above, we will test descendants of two selfed BC_1 plants from the BC_1_sat_FR population that were highly resistant (ISL 2 and 7) against *Bremia* (race Bl:21). Both BC_1 plants are heterozygote for the peak marker of the mid_C6 locus a marker closely related to the assumed QTL on C6, only one of them was heterozygote (genotype code: h) for a marker closely related to the assumed QTL on C1 and homozygote *L. sativa* (genotype code: a) on C7 (C1:h-C6:h-C7:a), the other was heterozygote for the marker closely related to the assumed QTL on C7 and homozygote *L. sativa* on C1 (C1:a-C6:h-C7:h). This additional segregation information and phenotyping could confirm the findings about interaction between the QTL at C6, and the QTLs on C1 and C7.

At the start of the thesis an initial experiment was performed to get familiar with the pathosystem. An F_1 progeny of a cross between *L. saligna* accession CGN05310 from Israel (ISR) and *L. sativa* cv. olof was tested for the first time in a disease assessment. The expectation is that these F_1 are highly resistant against *B. lactucae*. If so, this accession can also be used in the long term research activities in revealing the working of the non-host resistance of *L. saligna*. It is interesting to see whether there are differences between the three *L. saligna* accessions from Georgia, France and Israel in this mechanism.

Since it is relatively easy to score some other phenotypic characteristics in the BC_1_sat_GEO, like leaf colour, bolting, the presence of side shoots, leaf tip shape and other distinct phenotypes, this was done. Mapping these characteristics is interesting for lettuce breeding purposes as a trait or as a morphological marker.

2 Materials and Methods

2.1 Plant material

In previous research cultivated lettuce *L. sativa* cv. Olof (highly susceptible for *B. lactucae*) as father was crossed with three geographically distinct L. saligna accessions (all highly resistant for B. lactucae) as mothers, CGN15705, CGN05271 and CGN05310 that have been collected from Georgia (GEO), France (FR) and Israel (ISR) respectively (provided by the Centre for Genetic Resources, the Netherlands, http://www.cgn.wur.nl). F_1_GEO and F_1_FR plants were crossed with the cultivated parent to obtain BC_1sat_GEO and BC_1sat_FR seed. The ISLs (for Bl 21 and Bl 24) of the BC_1sat_FR were already determined in disease tests and the plants were selfed to obtain BC_1_satS_1_FR lines.

In this study a population of 65 BC_1sat_GEO plants and 23 plants from two BC_1_satS_1_FR (BC1sat_FR_51-pv11778.09 and BC1sat_FR_79-pv11781.19) lines were phenotyped in three disease tests and genotyped with a set of 79 markers. The F_2_BC_1sat_FR lines were selected due to the low ISLs of 2 and 7 of the mother plants (based on the mean ISLs of two experiments, both with Bl:21) and the presence of QTLs which are assumed to be involved in epistatic interactions (den Boer et al. 2014). In another disease test the F_1_ISR was tested.

For the disease tests, the following control lines were included as reference controls: for susceptibility: *L. sativa* cv. Olof, *L. sativa* cv. Cobham Green and BIL1.2; for intermediate: BIL8.2 and BIL1.2+8.2; and for strong resistance reactions: *L. saligna* GEO, *L. saligna* FR, *L. saligna* ISR, *L. sativa* cv. Olof and dBIL468.

Seeds were sown according standard procedure in moist soil for 3 days at 4° C and then moved to 20/16° C day/ night temperature under greenhouse conditions. At 3 to 4 weeks after sowing plants were transplanted to bigger pots and moved to a larger greenhouse compartment with 20/18 °C day/night temperatures. The sowing date for the F1_ISR plants (+ controls) was 6 September 2012, for the BC1sat_GEO plants (+ controls) 21 September 2012 and for the F_2_BC_1sat_FR plants (+ controls) 27 September 2012.

2.2 Genotyping

DNA was isolated from leaves from young plants (2-3 week old) with the KingFisher Plant DNA kit and a Flex Magnetic particle processor (Thermo Scientific, Waltham, USA). This was done for the BC_1sat_GEO, BC_1S_1sat_FR, and their original parents. DNA in elution buffer was stored in -20°C freezer as main stock, diluted DNA (5 to 40 ng/µl) was stored in 6°C fridge as working stock. A set of 82 EST-based markers randomly distributed on the genome was used for genotyping (see Appendix 1). The EST sequences were obtained in the Compositae Genome project (htttp://compgenomics.ucdavis.edu/).

For these markers reverse and forward primers were available (Appendix 1) and these were already tested en proven to be useful in genotyping populations descending form *L. saligna* and *L. sativa* hybrids (den Boer et al. 2014), in other words they had to work and to be polymorphic. With these primers PCRs were done on the isolated DNA. For each PCR 1 µl diluted DNA and 9 µl PCR mix was used. The PCR mix is together with the PCR program described in Table 1.

Table 1: PCR mix and PCR program in advance of high resolution melting curve analyses

PCR mix for 100 PCRs		PCR program		
volume	ingredients	temperature	time	
12.5 µl	Phire® Hot Start II [a]	98.0°C	30 sec	
200 µl	5x Reaction Buffer [a]	98.0°C	10 sec	40x
40 µl	dntp's 5 mM	60.0°C	10 sec	
560 µl	H_2O (MQ)	72.0°C	30 sec	
2.5 µl	fw primer 100 mM	72.0°C	30 sec	
2.5 µl	rev primer 100 mM	94.0°C	30 sec	
100 µl	10x LC Green™ Plus+ [b]	25.0°C	30 sec	

[a] Thermo Scientific, Waltham, USA
[b] BioFire Diagnostics, INC. , Salt Lake City, USA

Genotyping was done by high resolution melting curve analysis of amplicons obtained from these PCRs. The LightScanner® Sytem (BioFire Diagnostics, Inc. , Salt Lake City, USA) was able to measure the melting pattern of the amplicons due to the LCGreen fluoresent dye which binds to dsDNA. Polymorphisms between the parents gave a different melting pattern, visualized by a melting curve. The melting curves of individuals in the mapping population were compared, in the accompanying software, to those of the parental lines and the F_1 (F_1 DNA was artificially made by mixing DNA from both parents in equal proportions) to determine from which parent they inherited a SNP allele for the particular marker. For each marker each mapping individual was scored heterozygote (h) homozygote *L. sativa* (a) or homozygote *L. saligna* (b) or unknown (u) in case the melting curve didn't show a clear similarity to a specific genotype.

2.3 Phenotyping

2.3.1 B. lactucae resistance

A small young plant disease test (YDT) (Zhang et al. 2009) was performed on F_1_ISR plants. This was done in two transparent plastic cages in the greenhouse, in each cage a different *B. lactucae* race was used: Bl:21 and Bl:24. In each cage four F1_ISR plants, four *L. saligna*_ISR and four *L. sativa* cv. Olof were put for the YDT, these plants were sprayed by 10 ml in water soluted spores, 3 $*10^5$ spores/ml for BL21 and 2 $*10^5$ spores/ml for BL24. The first two days the plastic cages were covered by black plastic, to prevent UV radiation to inhibit the infection of the *B. lactucae* into the lettuce plants. After 7, 8, 9 and 12 dpi, the percentage leaf surface area covered by sporophores on the 5 oldest leaves of each plant were measured by eye (this is the ISL in the YDT). In the same cages trays with young *L. sativa* plants from cultivar Cobham Green and Olof were put for *B. lactucae* propagatation for later disease tests, these were also sprayed with 10 ml in water soluted spores. Sporophore covered leaves from the trays with CobhamGreen and Olof were put in plastic 50ml bluecap tubes and stored in -80°C freezer at 8 dpi for Bl21 and at 9 dpi for Bl24. These spores would later be used in the three disease tests mentioned here below.

Three Adult disease test with greenhouse plants (ADTg) (Zhang et al. 2009) were performed on the BC_1sat_GEO and the BC_1sat S_1_FR populations and all the control plants, the parents included. The first two ADTgs were done on the same day, 5 November 2012 for BC_1sat_GEO 45 dps, for BC_1sat S_1_FR 39 dps. In total eight plastic boxes were prepared with wet cotton wool and paper on top. On the paper, gridlines were drawn, to make cells which could be coded by row and column wherein leaf pieces could laid. In each box one leaf piece per plant should be laid. From each plant four full grown leaves were selected. From this leaves, square leaf pieces were cut with the approximate size of 1.5x2.5 cm without the main veins of the leaves. From each leaf two pieces were cut. So in total eight pieces were cut per plant. Four of them were assigned to be used for the ADTg with Bl:24 and the other four for the ADTg with Bl:21, the assignment was such that none leaf pieces of the same leaf were assigned to the same ADTg. The leaf pieces were put in the boxes with the lower epidermis above and sprayed with 20 ml in water soluted spores, 2.5 $*10^5$ spores/ml for BL21 and 2.9 $*10^5$ spores/ml for BL24. The boxes were put in transparent plastic bags to keep humidity high kept in a climate closet (15°C, 16L/8D) the first 16 hours a plastic cover protected the inoculation event from inhibiting UV-radiation. For each leaf piece the percentage off surface area covered by sporophores was observed (this is the ISL scale in the ADTg which went from 0 to 100%) at 7,8,9,10 and 11 dpi for BL24 and at 7,8,9 and 11 dpi for BL21. The third ADTg was almost the same as the second ADTg, except for the fact that this ADTg started two days later at 7 November 2012, the concentration of soluted spores was 3.0 $*10^5$ Bl:24 spores/ml in 20 ml water per box. Four boxes were used, again from each plants four leaf pieces were token from four different leaves and divided over the four boxes. Some of these leaves were used in the second ADTg and some were not. The percentage off surface area covered by sporophores was observed at 7,8, and 9 dpi for each leaf piece.

2.3.2 Morphological phenotypes

traits that are macroscopically visible and relatively easy to classify were monitored. The following phenotypic traits were monitored: early bolting, the presence of side shoots, leaf colour (the shade of green), the presence of anthocyan, the leaf tip shape and twisting leafs.

Early bolting was observed by measuring the main stem length from the surface of the potting soil. Early bolting is a trait associated with *L. saligna* and unwanted in a final cultivar. The same is valid for sideshoots which were of were not observed, and leaf colour which was classified in " dark green" the *L. saligna* phenotype and "light green" the *L. sativa* cv. Olof phenotype. Anthocyan was or was not observed, the leaf tip could be more *L. saligna* type which is point of more *L. sativa* type which is more round. Twisted leafs were or were not observed.

2.4 QTL mapping

The association of resistance and the morphological traits to specific loci was performed by a QTL analysis. Mapping of the QTLs was done by combining the genotypic data with the phenotypic in special developed mapping software. Kruskal-Wallistest (a non-paramatric test) and Interval Mapping on the data were performed with MapQTL 6 ® (Ooijen van 2009) and QTLnetwork 2.0 (Yang et al. 2008).

The manual of MapQTL 6 ® advices to use a significance threshold of α=0.005 for the test statistic of the Kruskal-Wallis test, since the test will be performed on the linked and unlinked loci without differentiating. According to the manual, a significance threshold of at least α=0.005 would obtain an significance of α=0.05 overall.

With a permutation test the corresponding LOD-score (test statistic) for an significance level of α=0.05 in interval mapping is determined, this was 2.6.

3. Results

3.1 Young Disease Test on F1_ISR and their parents

A Young Disease Test (YDT) was performed on the F1_ISR plants of almost 3weeks old (21 dps). The parental line *L. saligna* CGN05310 (ISR) behaved as expected with a 0.0 mean ISL for both races. The parental line *L. sativa* cv. Olof had for Bl:21 a lower mean ISL than expected. The YDTs in Figure 2. gave ISL higher than 90 for plants in this stage at 9 dpi. No sporulation was observed on the F1 plants at 9 dpi (table 2) and at 12 dpi against both races.

Table 2: Average ISLs at 9 dpi in the YDT on F1_ISR

Bl race	21		24	
	mean[a]	Std dev	mean[a]	Std dev
L. sativa cv. Olof	44.6	33.4	20.8	20.7
L. saligna CGN05310 (ISR)	0.0	0.0	0.0	0.0
F1_ISR	0.0	0.0	0.0	0.0

[a]mean ISL of 2nd,3rd and 4th leaf of 4 plants

3.2 Hybrid necrosis in progeny form *L. saligna* CGN05271 from France

All 23 BC_1_$satS_1$_FR plants were genotyped. In these plants hybrid necrosis was expected due to a locus auto-incompatibility disorder reaction between a (French) *L. saligna* locus RIN4 at 6.7 cM on C9 and two or more *L. sativa* loci near a marker (CLS_SR_Contig8361) at 34.4 cM on C6 (Jeuken et al. 2009)(Vullers 2012). This hybrid necrosis is associated with *B. lactucae* resistance. Based on original findings in Jeuken et al. 2009, three necrotic phenotypes are possible as follow: Plants which are homozygote *L. sativa* for the C6 locus and homozygote *L. saligna* for the C9 locus (C6:a-C9:b) are expected to be lethal, homozygote *L. sativa* for the C6 locus and heterozygote for the C9 locus (C6:a-C9:h) are expected to have a severe necrotic phenotype, those which are heterozygote for the C6 locus and homozygote *L. saligna* for the C9 locus (C6:h-C9:b) are expected to have a mild (low) necrotic phenotype, other genotypes are not expected to show a necrotic phenotype.

In total four plants from both BC_1_$satS_1$_FR populations had a genotype where severe hybrid necrosis was expected (C6:a-C9:h), all these four plants had severe hybrid necrosis. One of the plants even died in an early stage. All observed and expected hybrid necrosis phenotypes and the genotypes for the markers associated with the hybrid necrosis of these 23 plants are presented in Table 3. Four other plants had also a mild to severe form of hybrid necrosis, this was not expected for their genotype. Two of them were heterozygote for both loci (6h9h), one of them was homozygote *L. sativa* for both loci (6a9a) and one was heterozygote for the C6 locus and homozygote *L. sativa* for the C9 locus (6h9a) . These eight plants with hybrid necrosis were not used for the ADTg because it was known that the plants with hybrid necrosis gives higher resistance for *B. lactucae* due to auto-immunity (Jeuken et al. 2009). This could interfere with the observation of nonhost-resistance due to epistatic QTL interaction investigating of QTLs in resistance.

Table 3: Observed and expected hybrid necrosis in BC_1_$satS_1$_FR descendants and the genotype for the suspect loci (in bold) causing hybrid necrosis + genotype for the markers nearby.

	Marker	cM	parent 1	BC_1_$satS_1$_FR descendants parent 1												parent 2	BC_1_$satS_1$_FR descendants parent 2										
C6	LK1471	14.3	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
C6	CLX_S3_14099	28.2	a	a	a	a	a	a	a	a	a	a	a	a	a	h	a	h	a	a	b	a	h	h	h	a	a
C6	**CLS_S3_Contig8361**	**34.4**	**h**	**a**	**a**	**h**	**h**	**h**	**a**	**h**	**a**	**h**	**h**	**a**	**h**	**h**	**a**	**h**	**a**	**a**	**b**	**a**	**h**	**h**	**a**	**a**	**a**
C6	NL1117	50.0	h	a	a	a	h	h	a	h	a	h	h	a	h	h	a	h	h	a	h	a	a	h	h	h	a
C6	LE0178	57.7	h	h	u	a	h	h	a	h	u	h	u	u	a	h	b	h	h	u	h	u	a	u	h	a	h
C9	**RIN4_Snip_4**	**6.7**	**h**	**h**	**a**	**h**	**h**	**a**	**h**	**a**	**a**	**h**	**h**	**a**	**h**	**h**	**u**	**u**	**a**	**a**	**h**	**h**	**a**	**a**	**h**	**a**	**a**
C9	M431dCAPs	20.0	h	u	a	u	u	a	u	u	a	u	u	a	u	h	a	u	u	u	u	u	u	u	u	u	u
C9	CLX6726	31.1	h	h	a	h	h	a	h	h	a	h	h	u	h	h	a	h	a	a	h	h	a	a	h	b	a
	Expected phenotype*			2				2													2			2			
	Observed phenotype*			2				2		3		3		3							1-2		2-3	2			

* No phenotype notated means no necrotic phenotype expected or observed. In case of two phenotype codes are mentioned, the actually phenotype which was observed was intermediate.
genotypes: a=homozygote *L. sativa* | b=homozygote *L. saligna* | h=heterozygote | u=unknown
phenotypes: 1 = lethal | 2=severe hybrid necrosis | 3=mild (low) hybrid necrosis

3.3 Adult Disease Tests greenhouse

Three disease tests were performed on 63 plants from the BC_1sat_GEO population and on the remaining fifteen (non necrotic) F_2_BC_1sat_FR plants to gather phenotypic resistance data. The first (ADTg1) and the second (ADTg2) were performed on the same day, with Bl:24 and Bl:21 respectively. The third disease test (ADTg3) was started two days later with Bl:21. Per plant four leafs were chosen. Two leaf pieces per leaf were taken from which one was used in ADTg1 and the other in ADTg2. For ADTg3 some leaf pieces were taken form already used leafs in the previous two disease tests. and some from other not used leafs. This was done inconsequent. Infection severity levels (ISLs) were measured between 7 and 11 dpi for ADTg1 and ADTg2, for ADTg3 between 7 and 9 dpi.

Figure 5 gives the mean ISL per control line and populations for 9 dpi. For ADTg1 the mean ISLs are also presented for 11 dpi, since the used Bl:24 in ADTg1 is considered as a slowly developing and less virulent strain than Bl:21. For the susceptible control lines *L. sativa* cv. Olof and cv. Cobham Green the difference in ISL between ADTg1 and the other two ADTgs (at 9 dpi) was smaller on 11 dpi than on 9 dpi. Therefore the decision was made to use the ISLs of 11 dpi for ADTg1 and the ISLs of 9 dpi of ADTg1 and ADTg2 for mapping *B. lactucae* resistance.

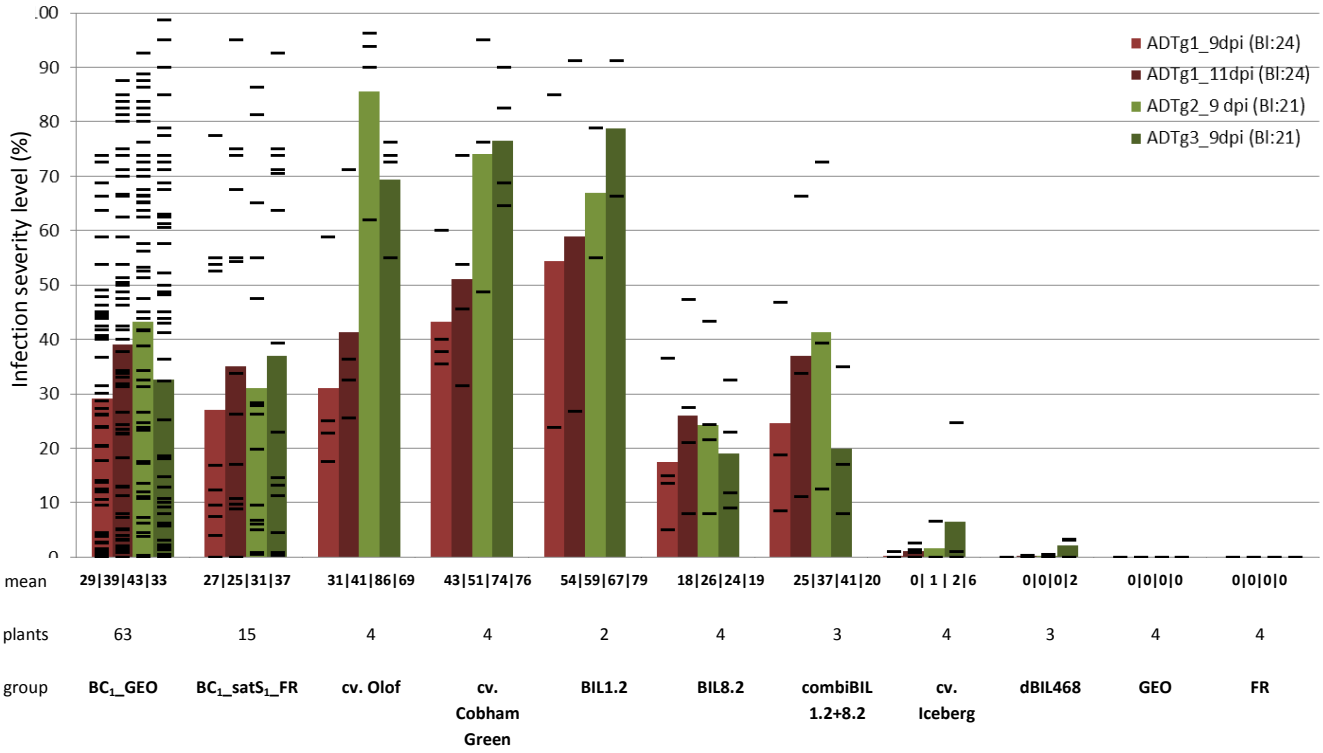

Figure 5. Infection severity plots of the populations and control lines tested in three ADTgs. The ISLs of ADTg1 (Bl:24) are both presented for 9 and 11 dpi, for ADTg2 and ADTg3 (BL:21) only for 9 dpi. Each data point is the mean ISL of 4 leafs of a single plant. The bars in this plot represent the mean ISLs of the populations and control lines per ADTg on a specific moment.

Correlations between the ADTgs at 9 dpi are presented in Table 4. For ADTg1 both the ISLs of 9 and 11 dpi are used in the correlation calculations. The disease tests performed on the same day, ADTg1

and ADTg2, have the strongest correlation with a correlation coefficient (r) of 0.77 for 9 dpi and 0.76 for 11 dpi. based on the ISLs of all tested plants. The disease test with the same *B. lactucae* strain, ADTg2 and ADTg3, are almost as strong correlated with a r of 0.74. The lowest correlation is found between the disease tests performed on a different day and with a different *B. lactucae* strain, ADTg1 and ADTg3, the r is 0.53 for 11 dpi and 0.55 for 9 dpi.

The correlations between the ADTgs are also calculated for selective groups of the tested plants. for the control lines. for the BC_1sat_GEO and for F_2_BC_1sat_FR. The correlations coefficients between the ADTgs are for the ISLs of the BC_1GEO the lowest of the three groups. The strongest correlations between the ADTgs are for the ISLs of the control lines. The r's between the ADTgs for F_2_BC_1sat_FR gave values in between. Figure 6 visualises the correlation between ADTg2 and ADTg3 for all the ISLs and for the ISLs of BC_1GEO and the control lines.

Table **4**: Correlation between the ADTgs described with the Pearsons correlation coefficient (r)

Data		ADTg1_9dpi	ADTg1_11dpi	ADTg2_9 dpi	ADTg3_9dpi
all 110 plants	ADTg1_9dpi	-	0.97	0.76	0.55
	ADTg1_11dpi		-	0.77	0.53
	ADTg2_9 dpi			-	0.74
	ADTg3_9dpi				-
Control lines 32 plants	ADTg1_9dpi	-	0.99	0.86	0.82
	ADTg1_11dpi		-	0.90	0.81
	ADTg2_9 dpi			-	0.91
	ADTg3_9dpi				-
BC_1sat_GEO 63 plants	ADTg1_9dpi	-	0.97	0.67	0.37
	ADTg1_11dpi		-	0.69	0.37
	ADTg2_9 dpi			-	0.67
	ADTg3_9dpi				-
BC_1_$satS_1$_FR 15 plants	ADTg1_9dpi	-	0.98	0.90	0.69
	ADTg1_11dpi		-	0.84	0.65
	ADTg2_9 dpi			-	0.76
	ADTg3_9dpi				-

Bl:24 used for ADTg1, Bl:21 used for ADTg2 and 3

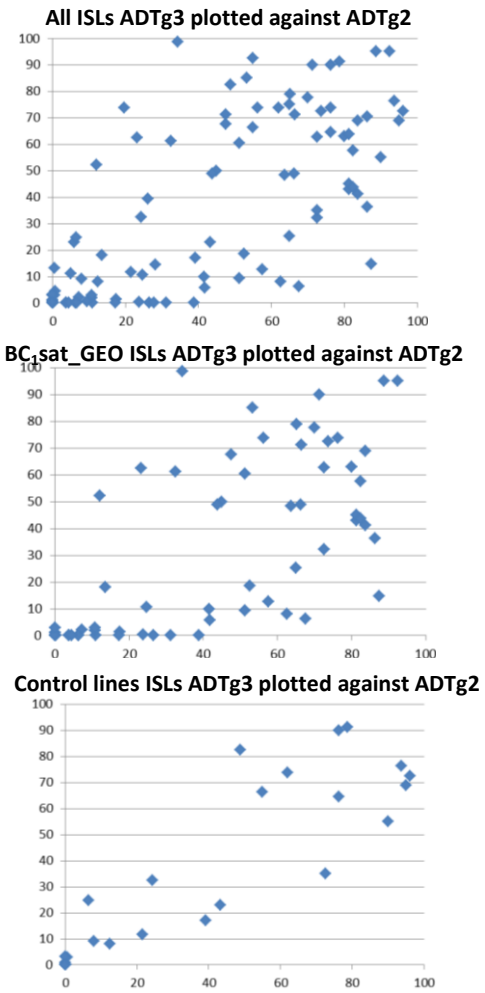

Figure 6. Correlations between ADTg 2and ADTg3 visualised for all plants (r=0.74), the BC_1sat_GEO (r=0.67) and the control lines (r=0.91)

3.4 Four F_2_BC_1sat_FR plants found where epistasis is expected

The remaining fifteen plants were phenotyped in the ADTgs. Unfortunately there was not a single plant with a *L. saligna* allele on both the loci of the locus couple mid C6 and mid C7, which are suspect for epistasis, with significant decrease in ISL. There were four plants with at least one *L. saligna* allele on both loci bot C1 and mid C6, which are suspected to be in epistasis, with significant decrease in ISL. Two of them had a low ISL (5 and 15) and two had high ISL (62 and 65). While there were also two plants with low ISL (5 and 5), which had no *L. saligna* allele on mid C6. The genotypes for the suspect epistatic markers and the corresponding phenotypes for the fifteen plants are presented in Table 5.

Table 5: The genotype for the makers (in bold) linked to the loci suspected to be involved in epistatic interactions and the flanking makers of the BC_1_$satS_1$_FR plants which were phenotyped

	marker	cM	parent 1	BC_1_$satS_1$_FR descendants parent 1						parent 2	BC_1_$satS_1$_FR descendants parent 2								
C1	NL0267	73.5	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
C1	**QGF24O17**	85.4	a	a	a	a	a	a	a	h	b	b	b	b	a	a	b	b	a
C1	QG_CA6637	87.6	a	a	a	a	a	a	a	h	b	b	b	b	a	a	b	b	a
C6	NL1117	50.0	h	h	a	a	h	h	a	h	a	h	h	h	a	h	a	a	h
C6	**LE0178**	57.7	h	h	u	a	h	h	u	h	a	h	a	h	u	h	b	h	h
C6	LE01126	66.3	h	a	a	a	a	a	a	h	a	h	a	h	a	a	a	h	h
C7	NL1034	33.6	h	u	a	h	u	h	a	a	a	a	a	a	a	a	a	a	a
C7	**NL0650**	47.0	h	a	a	u	u	u	u	a	a	a	a	a	a	a	a	a	a
C7	LK1513	54.9	h	a	a	h	h	a	h	a	a	a	a	a	a	a	a	a	a
		ISL*	7	3	5	26	34	67	74	2	5	5	5	15	25	40	62	65	84

*a mean ISL based on two ADTgs with Bl:21

genotypes: a=homozygote *L. sativa* | b=homozygote *L. saligna* | h=heterozygote | u=unknown

3.5 Skewed segregation and F_2's found during genotyping BC_1sat_GEO

In total 72 seeds were sown originating form three F_1_GEO mother plants which were backcrossed with *L. sativa* cv. Olof. Seven of the young plants were phenotypically distinct from the rest. these plants were clearly not the result of a backcross, but F_2 progeny due to unwanted selfing, what is observed regularly in cross-pollinating lettuce by hand. These plants were discarded. Genotyping the remaining plants revealed that two other plants were homozygote *L. saligna* for several markers on more than one chromosome. These plants were not used in further analysis since these plants were F_2 plants as well. Phenotypic data of these F_2's was not presented in the paragraphs here above, all phenotypic and genotypic data presented in this chapter about the BC_1sat_GEO is the data of the remaining 63 true BC_1sat_GEO plants.

Genotyping of the BC_1sat_GEO population was done successfully with 77 markers containing a polymorphism. Originally 82 markers were used. but five were not informative with the HRM-curve analyses. Looking at the segregation for each marker. Mendelian segregation (a:h=1:1) was true for most markers. but not for all. see Figure 7. The markers in the end of C9 gave the largest deviation. The observed *L. saligna* allele frequency of was less than 0.1 in this region, while 0.25 expected. This resulted in only 10 heterozygotic plants for the last marker CLS_S3_4656 at 110.4 cM. Two plants were scored unkown and 51 homozygote *L. sativa*.

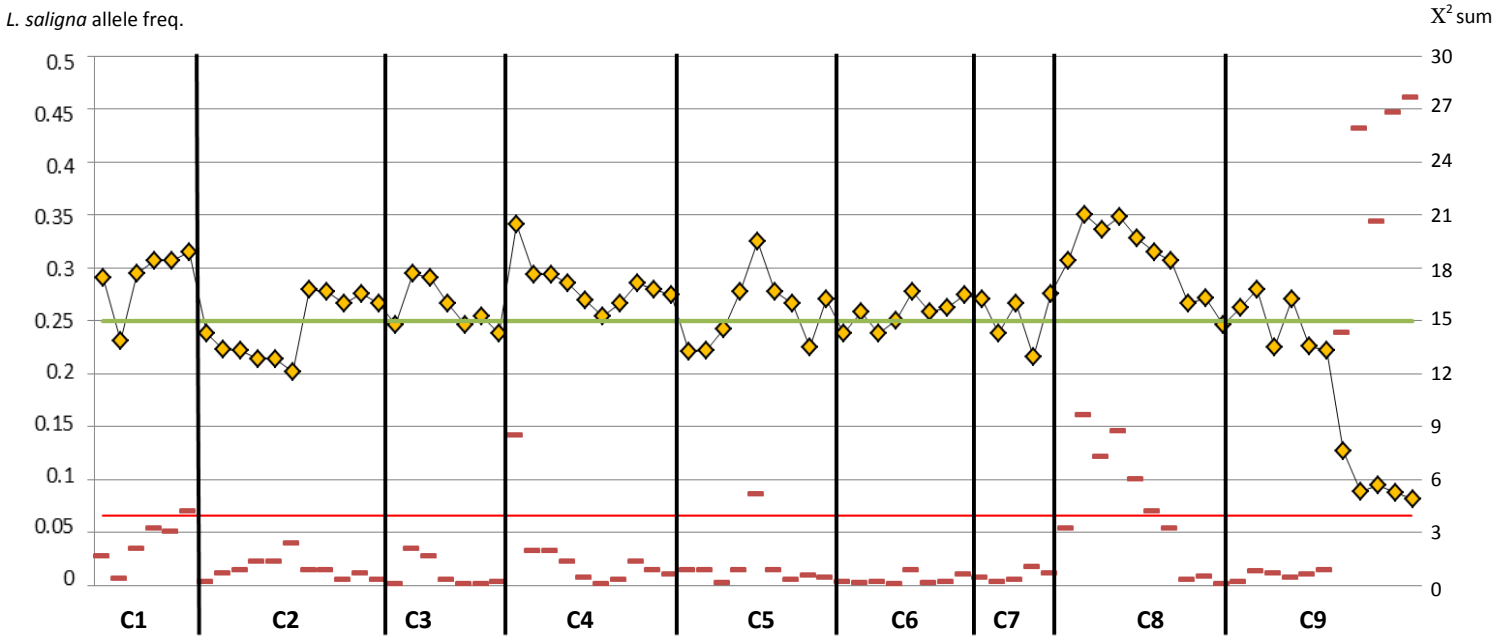

Figure 7. The *L. saligna* allele frequency and segregation ratio deviations from Mendelian are shown per marker for the BC1sat_GEO (n=63, two non-phenotyped individuals not included in the data). The *L. saligna* allele frequency per marker is represented by a yellow diamond. The saligna allele frequency of 0.25 for normal segregation is shown as a green line. The red stripes gives the X^2 value for segregation ratios per marker. When these values are above the tresholdline (in red at 3.84) than the distribution between heterozygotes and homozygote *L. sativa* is significant not Mendalian (α=0.05). Remark: The total number of a+h genotypes varied per marker due to the fact that some individuals were scored as unkown (u), The average number of u's per marker was 2.1. Extreme number of u's were found at five markers with each 10 to 13 u's scored for in total 63 inidividuals.

3.6 B. lactucae resistance mapping with MapQTL 6

With Interval mapping and the Kruskal-Wallis test in MapQTL 6® *B. lactucae* resistance QTLs were predicted on C4. C7. C8 and C9. The test statistics for both test on these four chromosomes are plotted against the maker positions in Figure 8 until 11. The predicted QTL with a peak marker on C4 at 48.9 cM was only significant for ADTg1 and only for the Kruskal-Wallis test. Interval mapping gave a peak for ADTg1 at the same position for ADTg1 just below the significance threshold line. The QTL with a peak marker on C7 at 47.0 was only significant for ADTg2. for both the Kruskal-Wallis test and Interval mapping.

For the QTLs predictions on C8 and C9 the peaks between the interval mapping and Kruskal-Wallis test differ in one marker position. The peak marker for the predicted QTL on C8 for the Kruskal-Wallis test is positioned on 37.7 cM and is significant for both ADTg2 and ADTg3. while interval mapping gave the peak marker at 30.2 cM on C8 significant for ADTg3. and just below the significance threshold line for ADTg2. The peak marker for the predicted QTL on C9 for the Kruskal-Wallis test is positioned on 107.0 cM. and for interval maping at 106.0 cM. in both statistical tests significant for all three ADTgs.

The effect and the explained percentage of variance of the *L. saligna* allele for each *B. lactucae* peak marker per ADTg is given in Table 6. Each effect is negative for the ISL. which is off course positive for the resistance against *B. lactucae*.

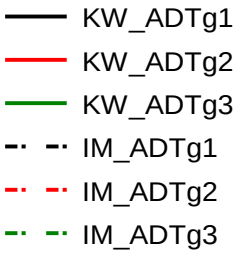

Figure 7. Legenda for Figures 8 to 11.
Horizontal line is treshold LOD=2.6 ~ α=0.05 and K=7.9 ~ α=0.005
KW = K-values (test-statistic) Kruskal-Wallis test
IM = LOD scores (test-statistic) Interval Mapping

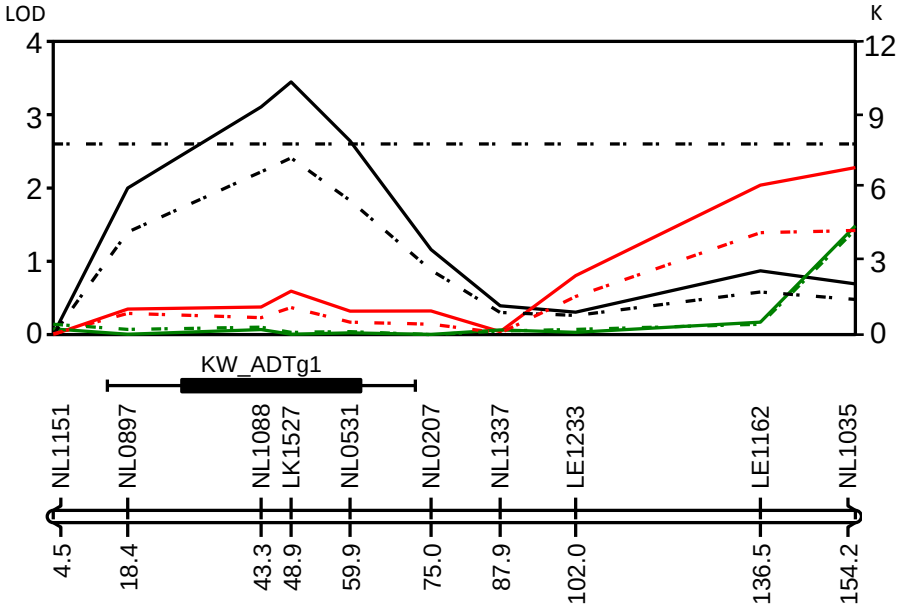

Figure 8. Test statistic Kruskal-Wallis test and Interval Mapping plotted against the marker positions in mapping *B. lactucae* resistance on Chromosome 4. See for legenda Figure 7.

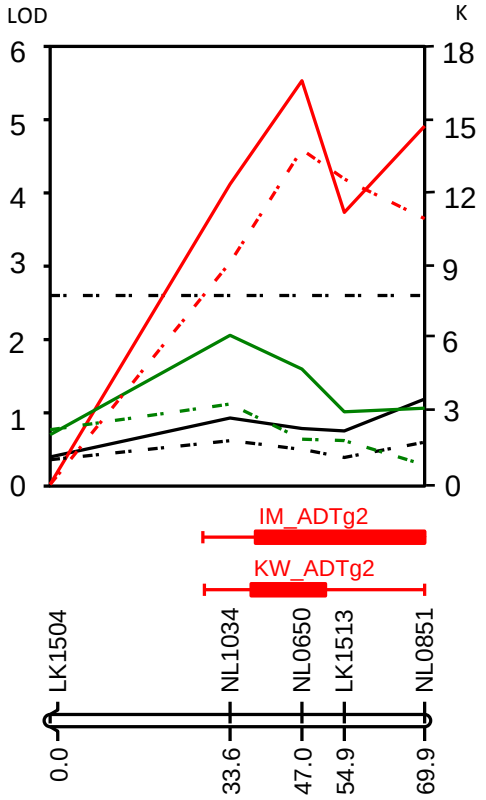

Figure 9. Test statistic Kruskal-Wallis test and Interval Mapping plotted against the marker positions in mapping *B. lactucae* resistance on Chromosome 7. See for legenda Figure 7.

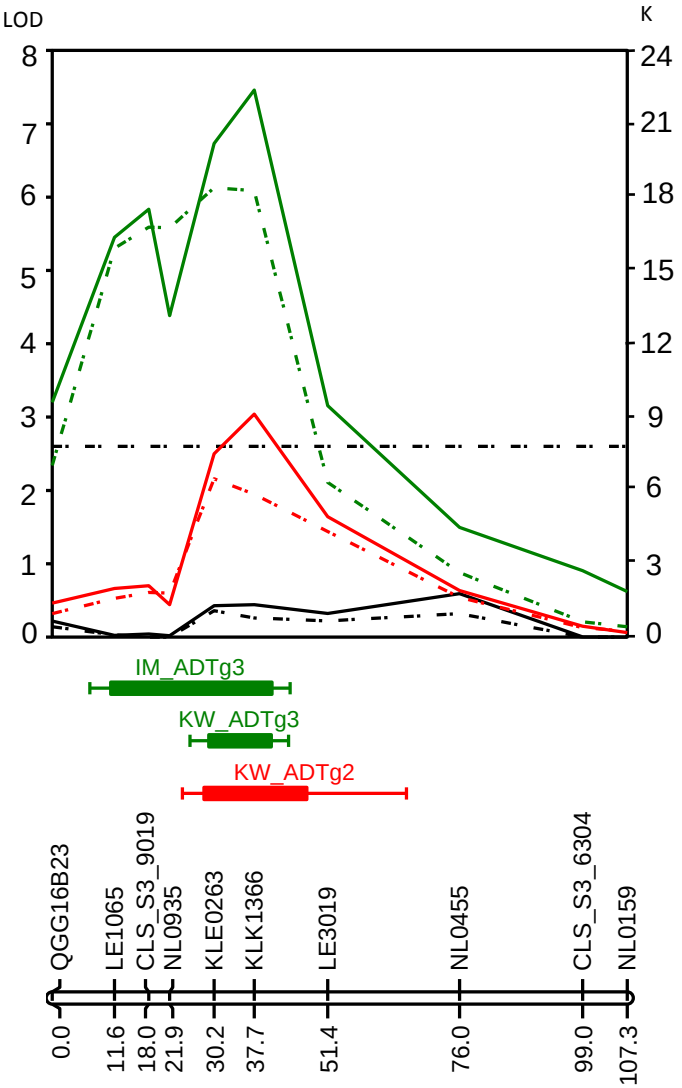

Figure 10. Test statistic Kruskal-Wallis test and Interval Mapping plotted against the marker positions in mapping *B. lactucae* resistance on Chromosome 8. See for legenda Figure 7.

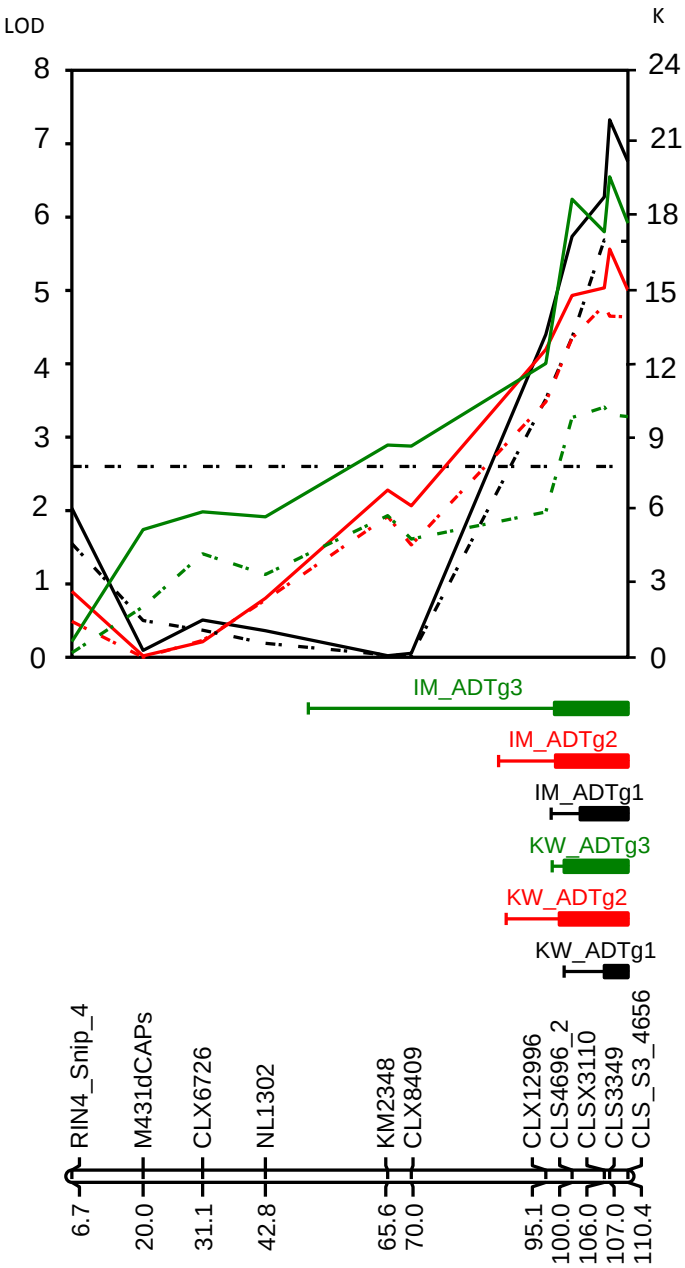

Figure 11. Test statistic Kruskal-Wallis test and Interval Mapping plotted against the markerpositions in mapping *B. lactucae* resistance on Chromosome 9. See for legenda Figure 7.

Table 6. The effect of the *L. saligna* allele on the ISL (µh-µa) and the percentage explained variance (expl) for the *B. lactucae* QTL peak markers

			ADTg1		ADTg2		ADTg3	
peak marker*	position (cM)		µh-µa	expl	µh-µa	expl	µh-µa	expl
KW LK1527	C4	48.9	**-22,5**	16.1	-10,0	2.6	2,9	0.2
IM&KW NL0650	C7	47.0	-10,7	3.6	**-32,7**	28.5	-14,1	4.6
KW KLK1366	C8	37.7	-8,2	1.9	**-25,0**	13.3	**-42,1**	35.9
IM KLE0263	C8	30.2	-8,4	2.6	-23,1	14.6	**-39,7**	36.1
KW CLS3349	C9	107.0	**-42,5**	34.0	**-42,9**	28.8	**-39,3**	21.5
IM CLSX3110	C9	106.0	**-42,3**	34.0	**-42,6**	29.6	**-39,5**	22.1

*significant *B. lactucae* QTL peak maker for the ADTgs with the effect in bold, KW only for Kruskall Wallis test, IM only for Interval Mapping.

3.7 B. lactucae resistance mapping with QTLnetwork 2.0

Mapping *B. lactucae* resistance with QTLnetwork gave only a significant peak for the QTL mapped with MapQTL6 at C7 (only for ADT_g2). For the QTLs found on C4 and C8, QTLnetwork2.0 gave peaks, but these were just not significant according to the sifnificant threshold the software has set. No peaks at all were observed at C9 in QTLnetwork2.0.

3.8 Mapping early bolting with MapQTL6

Two QTLs associated with early bolting were predicted with the Kruskal-Wallis and Interval Mapping in MAPQTL 6. one on C7 and one on C9. The QTL on C9 was just significant in the data of 46 dps. And not significant at all for the later measure moments. The QTL found on C7 was strong significant with all data sets, for 46 dps, 55 dps and 63 dps. The peak marker differs between Kruskal-Wallis test and Interval mapping. with one marker position. The test statistics for both test on these two chromosomes are plotted against the maker positions in Figure 13 and 14.

The effect and the explained percentage of variance of the *L. saligna* allele for each bolting peak marker per ADTg is given in Table 7. Each effect is positive for the stem length.

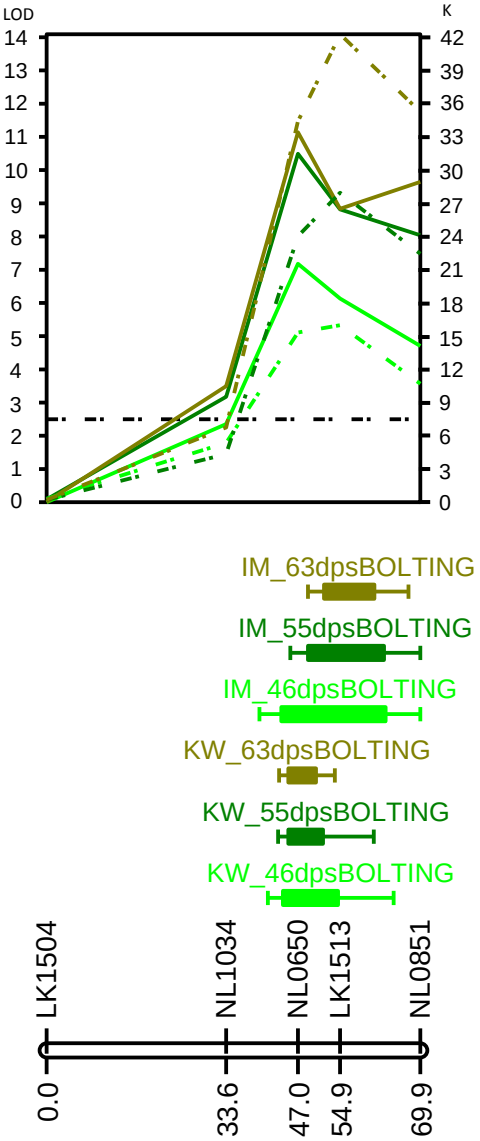

Figure 13. Test statistic Kruskal-Wallis test and Interval Mapping plotted against the marker positions in mapping early bolting on Chromosome 7. See for legenda Figure 12.

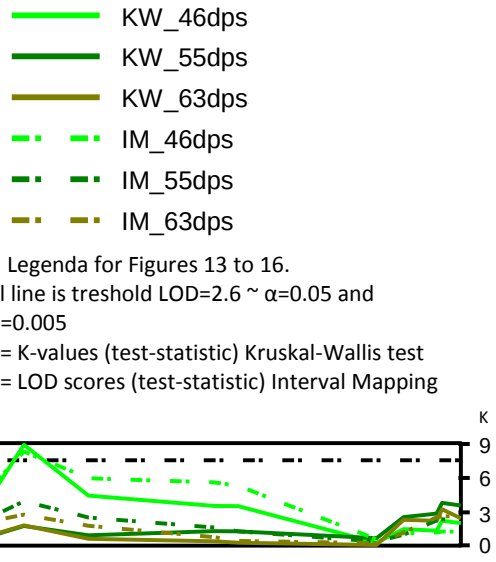

Figure 12. Legenda for Figures 13 to 16.
Horizontal line is treshold LOD=2.6 ~ α=0.05 and K=7.9 ~ α=0.005
KW = K-values (test-statistic) Kruskal-Wallis test
IM = LOD scores (test-statistic) Interval Mapping

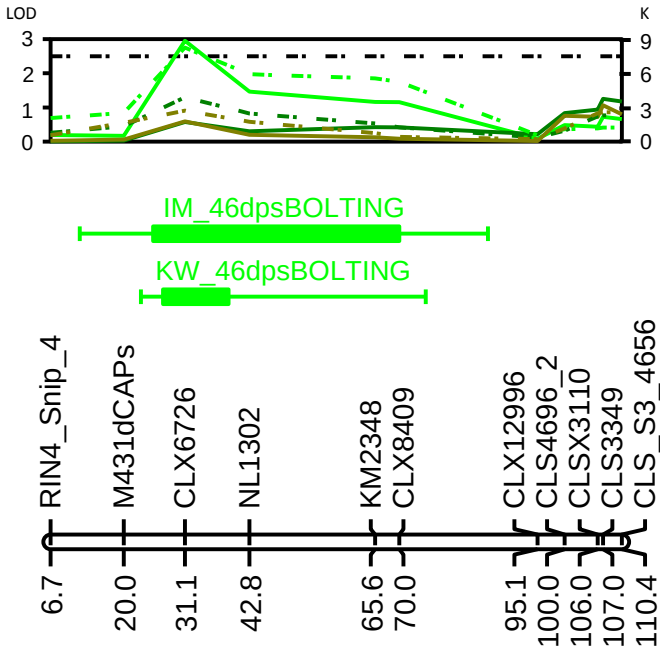

Figure 14. Test statistic Kruskal-Wallis test and Interval Mapping plotted against the marker positions in mapping early bolting on Chromosome 9. See for legenda Figure 12.

Table 7: The effect of the *L. saligna* allele on the main stem length (cm) and the explained variance (expl) for the early bolting QTL peak markers

			46 dps		55 dps		63 dps	
peak marker*	postion (cM)		µh-µa	expl	µh-µa	expl	µh-µa	expl
^{KW}NL0650	C7	47.0	**4.4**	31.2	**20.7**	44.3	**25.2**	56.7
^{IM}LK1513	C7	54.9	**4.5**	32.2	**22.6**	49.4	**26.5**	64.3
$^{IM\&KW}$CLX6726	C9	31.1	**3.5**	18.2	9.7	7.1	8.6	6.4

*significant bolting QTL peak maker for the with the effect in bold. KW only for Kruskall Wallis test. IM only for Interval Mapping.

3.9 Mapping the presence of side shoots with Map QTL6

Two QTLs associated with the presence of side shoots were predicted with the Kruskal-Wallis and Interval Mapping in MAPQTL 6. One on C7 and one on C9. The QTL on C9 was just significant in the data of 46 dps. And not significant at all for the later measure moments. The QTL found on C7 was strong significant with all data sets, for 46 dps, 55 dps and 63 dps. The peak marker differs between Kruskal-Wallis test and Interval mapping. with one marker position. The test statistics for both test on these four chromosomes are plotted against the maker positions in Figure 14 and 16.

The effect and the explained percentage of variance of the *L. saligna* allele for each peak marker per ADTg is given in Table 8. Each effect is positive for the presence of side shoots.

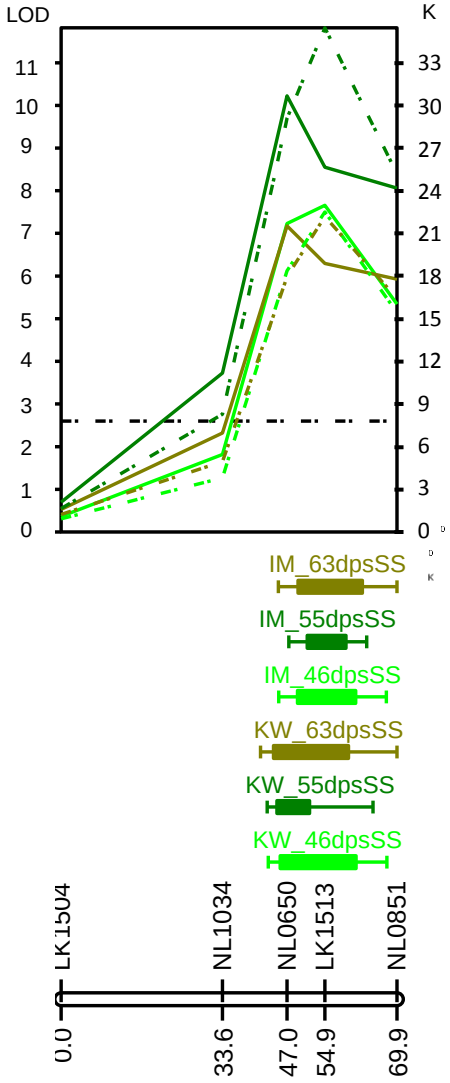

Figure 15. Test statistic Kruskal-Wallis test and Interval Mapping plotted against the marker positions in mapping the presence of side shoots on Chromosome 7. See for legenda Figure 12.

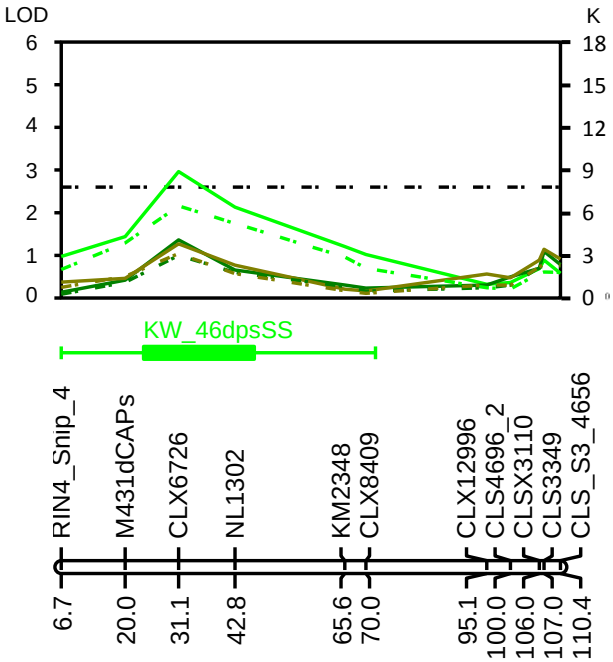

Figure 16. Test statistic Kruskal-Wallis test and Interval Mapping plotted against the marker positions in mapping the presence of side shoots on Chromosome 9. See for legenda Figure 12.

Table 8: The effect of the *L. saligna* allele on the fraction of plants with (1= with. 0 is without) sideshoots and the explained variance (expl) for the sideshoot QTL peak markers

			46 dps		55 dps		63 dps	
peak marker*	postion (cM)		µh-µa	expl	µh-µa	expl	µh-µa	expl
^{KW}NL0650	7	47.0	0.59	36.1	**0.71**	50.8	**0.56**	35.4
^{IM}LK1513	7	54.9	**0.66$_{+KW}$**	42.2	**0.70**	57.9	**0.61**	41.8
^{KW}CLX6726	9	31.1	**0.39**	14.6	0.27	7.1	0.24	7.3

*significant sideshoot QTL peak maker for the measuring moments with the effect in bold. KW only for Kruskall Wallis test. IM only for Interval Mapping.

3.9 Mapping leaf colour with Map QTL6

The leafs of *L. sativa* cv. Olof have a much lighter shade of green than the leafs of *L. saligna* GEO which have much darker green leafs. Furthermore, the red glow of anthocyanin can be observed in *L. saligna*. Mapping the anthocyanin in BC_1sat_GEO gave no significant association with a marker. The shade of green gave a QTL on C4 predicted with the Kruskal-Wallis and Interval Mapping in MAPQTL 6 (see Figure 17). The peak marker is NL0531 located at 59.9 cM and had an effect (µh-µa) of 1,2 (scoring: 0=light green; 1=in between; 2= dark green) and explained 43.3 % of the variance.

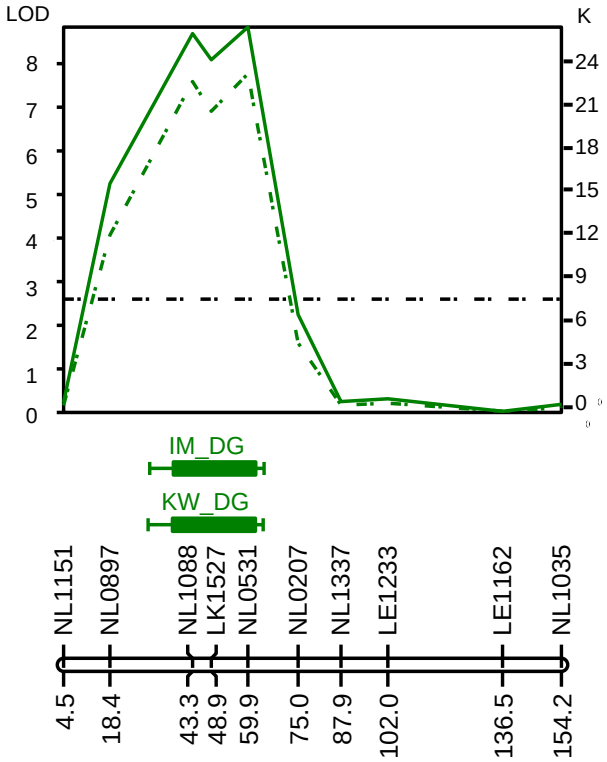

Figure 17. Darkgreen QTL predictions on Chromosome 4

3.10 Mapping leaf tip shape with Map QTL6

Two QTLs associated with the leaf tip shape were predicted with the Kruskal-Wallis and giving just non-significant peaks with Interval Mapping in MAPQTL 6, one on C3 and one on C7 (See Figure 18 and 19).

The effect and the explained percentage of variance of the *L. saligna* allele for each peak marker per ADTg is given in Table 9. Each effect is positive for the presence of side shoots.

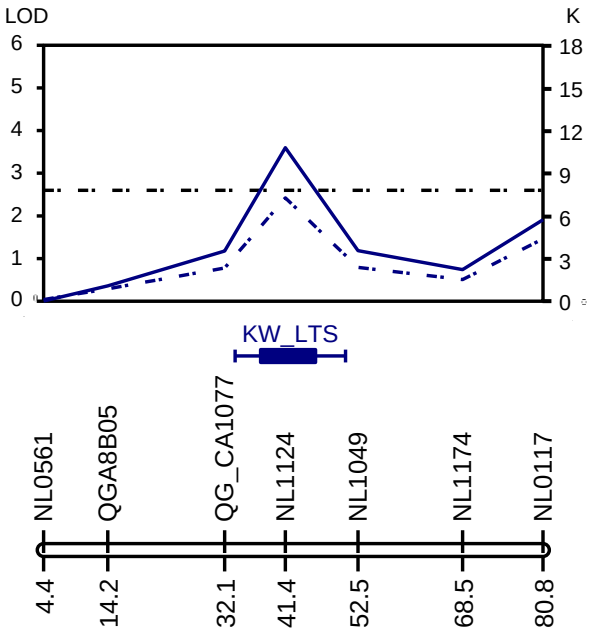

Figure 18. Round leafs QTL predictions on Chromosome 3

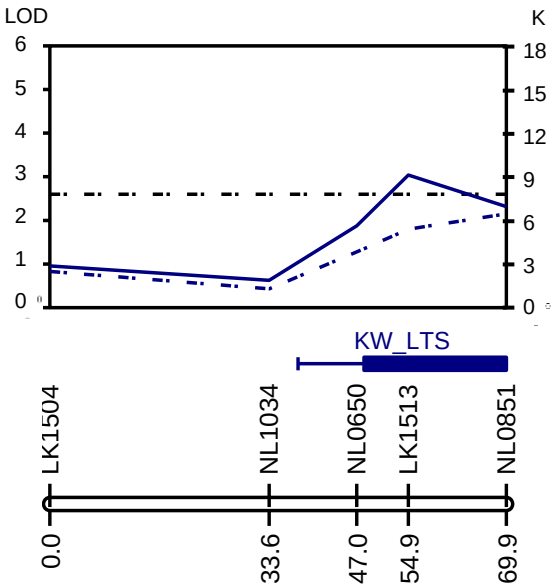

Figure 19. Round leafs QTL predictions on Chromosome 7

Table 9: The effect of the *L. saligna* allele on leaf tip shape (0=pointy; 1=pointy (bit round); 2= in between; 3=round (bit pointy); 4=round) and the explained variance (expl) for the sideshoot QTL peak markers

peak marker*	postion (cM)		µh-µa	expl
^{KW}NL1124	3	41.4	**-1.4**	16.2
^{KW}LK1513	7	54.9	**-1.5**	12.3

*significant sideshoot QTL peak maker for the measuring moments with the effect in bold. KW only for Kruskall Wallis test.

3.11 Mapping twisted leafs with Map QTL6

Another phenotype observed in the BC_1sat_GEO was the appearance of twisted leafs. This was not observed in one of the parents. In an attempt to map it, a significant QTL was predicted with Kruskal-Wallis test in MapQTL 6 (see Figure 20). The peak marker is LE1162 located at 136.5 cM and had an effect (µh-µa) of -0.62 (scoring amount of twisted leafs: 0=none; 1=a few; 2=around half; 3=almost all), and explained 14.2 % of the variance. An negative effect means that it is associated with the *L. sativa* allele.

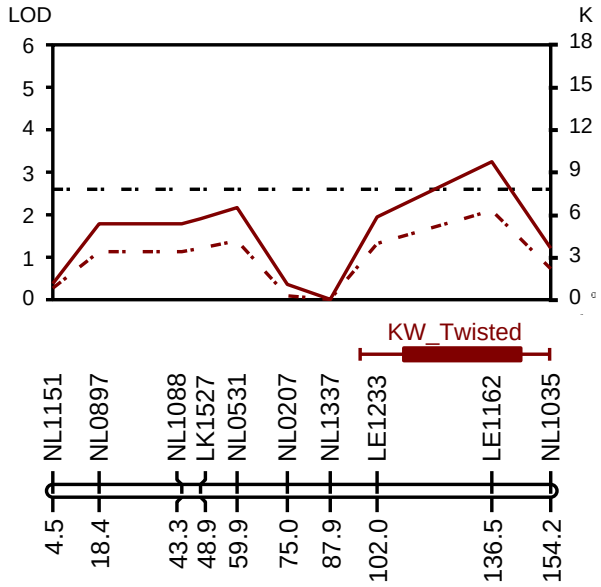

Figure 20. Twisted leafs QTL predictions on Chromosome 4

4 Discussion

4.1 *L. saligna* CGN05310 (ISR) is a potential source for *B. lactucae* resistance

The small YDT performed on F1_ISR and ISR plants with Bl:21 and Bl:24 gave ISLs of 0%. This makes this accession a potential source of *B. lactucae* resistance for cultivated lettuce, with the important note that this was a small scale experiment with a bit weaker disease pressure than in similar YDT experiments done before (see Figure 2, in Introduction). It is very likely that as was found in other *L. saligna* accessions (Jeuken and Lindhout 2002; Jeuken et al. 2008; den Boer et al. 2014), that a set of several QTLs is responsible for the non-host resistance, but it is not impossible that a single strong R-gene is responsible for a big part of the resistance. A continuous ISL segregation pattern in the F_2 would confirm the presence of several resistance QTLs.

4.2 Possible explanations of the unexpected hybrid necrosis

Four plants had a hybrid necrotic phenotype while this was not expected based on their genotypes for the markers associated with the two loci auto-immune response causing this phenotype. The *Rin4* gene from *L. saligna* (transcript: *Rin4salT2*) on C9 was in a previous study identified to cause hybrid necrosis with a locus on C6 (Jeuken et al. 2009). In another study it was revealed that at least two different loci on C6, one above and one beneath marker CLS_S3_8361 at 34.4 cM (Vullers 2012).

Two of the four unexpected necrotic plants had no *L. saligna* allele on the *Rin4* locus, which could indicate that there is another auto-immune response causing hybrid necrosis. The other two unexpected plants were heterozygote on the C6 marker CLS_S3_8361 and heterozygote for the *Rin4* locus. One marker beneath CLS_S3_8361, on marker CLX_S3_14099 at 28.2 cM these plants were homozygote *L. sativa*. The *L. sativa* loci on C6 involved in the Hybrid necrosis are not identified and localized precisely. So it could be that at least one involved loci is present between CLS_S3_8361 and CLX_S3_14099. And that the genotype for this locus for the last two mentioned plants is homozygote *L. sativa*. That would explain the observed necrosis response.

4.3 Strong correlations for the controls between ADTgs

It is remarkable that the correlations between the ADTgs for only the control line data is so high, while for the rest of the data these correlations are lower. An indication that the ISL scoring of the control lines was perhaps not done completely unbiased. This makes sense since, every time before scoring the most susceptible control lines were checked to see if the ISL was increasing every day as expected at the susceptible control lines. Furthermore the leaf pieces of the parental (control) lines and the cv. Iceberg were also very distinctive from the rest in colour and shape. Therefore it could be that between ADTgs the ISL differences of control lines are a bit smaller due to unknowingly scoring the control lines with expectations. This is unwanted in scientific research. It is however unlikely that it did influence the mapping the traits, since all the leaf pieces of BC_1sat_S_1_FR and BC_1sat_GEO looked very similar in the disease tests.

4.4 Epistasis between *B. lactucae* QTLs not confirmed in this thesis

Epistasis between earlier found resistance QTLs mid C6 and mid C7 and between bot C1 and mid C6 (erik boer H4 ref) were not confirmed with the two tested BC_1_$satS_1$_FR populations. If the populations were bigger in number we might have found some more non hybrid necrotic individuals which had at least one *L. saligna* allele on both the loci of the assumed interaction loci, and which did not fail in genotyping.Not a single plant was found with a *L.* saligna allele on both the C6 and C1 alele. The parent was heterozygote for both loci. So it was expected that 9 out of 16 BC_1_$satS_1$_FR would have a *L. saligna* allele on both loci. But here were only 6 plants without hybrid necrosis, and 4 of these failed to genotype somehow. Only four plant were available with *L. saligna* aleles for the mid C6 and mid C7 interaction and none for the mid C6 and bot C1. Two of the plants with a *L. saligna* on mid C6 and mid C7 gave high ISLs, which was not expected. It could be that other epistatic interactions are involved explaining these unexpected high ISLs.

4.5 Four *B. lactucae* resistance QTLs were mapped in this thesis which were mapped before.

The QTL found on the on the top of C4 (peak marker at 48.9 cM) is located in a region of BIL4.2, which contains a *L. saligna* introgression of 43 cM(Jeuken and Lindhout 2004). BIL4.2 which is tested in multiple disease tests with Bl:14 and Bl:16, including six ADTgs, this gave an average ISL decrease of 63% in the ADTgs when comparing it with susceptible parent *L. sativa* cv. Olof (Zhang et al. 2009). This QTL was only significant for ADTg1, the disease test with with Bl:24. It would be remarkable if this would be a race-specif resistance QTL, since this is not expected in non-host resistance. So maybe it is not non-host resistance, or it all QTLs together, including a few race-specific determine the non-host status of *L. saligna*.

The QTL found on the top of C8 (peak marker at 30.2 – 37.2 cM) is located in a region of BIL8.2 which contains a *L. saligna* introgression of 34 cM (den Boer et al. 2013). BIL 8.2 is as BIL 4.2 associated with *B. lactucae* resistance. Significant peaks were only found for Bl:21 and not for Bl:24, another case of race-specificity?

The QTL found on the middle of C7 (peak marker at 47.0 cM), was found before in selF$_2$_GEO and selF$_2$_FR with Bl:21 (den Boer et al. 2014) and in F$_2$_FR_1997 with Bl:14 and Bl:16 ((Jeuken and Lindhout 2002). This significant peak was only found for ADTg2, and not with the others two disease tests. The reason why ADTg1 with Bl:24 doesnot shows a peak, could be again due to race specificity. It is strange that ADTg3, which is actually the same disease test as ADTg2, did not give the peak. Since three independent mapping attempts with two different accessions (GEO and FR) as resistance source gave all three a *B. lactucae* QTL prediction near this locus it is very likely that there is something there.

The QTL found on the bottom of C9 (peakmarkers at 106 and 107 cM) was only earlier for an other earlier attempt with same resistance source, *L. saligna* GEO, and not in the ealier attempts with the other resistance source *L. saligna* FR. In this thesis both a peak was found for Bl:21 and Bl:24.

5 Conclussions

- Two *B. lactucae* resistance QTLs were mapped on loci which also belong to *L. saligna* introgressions of Backcross Inbred Lines which are associated with *B. lactucae* resistance.
- One *B. lactucae* resistance QTL was mapped on C9, on the position a QTL was mapped before for selF$_2$_GEO. This QTL appears to be absent in the France *L. saligna* accession since it was not predicted in selF$_2$_FR, F$_2$_1997 and
- The *B. lactucae* resistance QTL on C4 appears to be race-specific for Bl:24, and the QTL on C8 appears to be race-specif for Bl:21
- The QTL on C7 for early bolting was found before in selF$_2$_FR, selF$_2$_GEO and BC$_1$_GEO
- The QTL for Dark Green leafs on C4, was found in BIL 4.2 which has a *L. saligna* introgression which includes this QTL

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7 Apendices

Apendix 1 Marker set used for genotyping

The marker set used for genotyping was already used for mapping *B. lactucae* resistance from *L. saligna* by PhD candidate Erik den Boer(den Boer et al. 2014). The primers used in for this markers set were therefore already tested for being polymorphic between *L. sativa* cv. Olof and the *L. saligna* accessions from France and Georgia. Nevertheless a few primers failed to produce amplicons which gave informative high resolutions curves see Table A1.

Table A1. Primer sequences, contig and map position of the marker set used in this thesis.

Marker name	Contig or publication	Chr.	Position (cM)	Primer foreward	Primer reverse
LK1549	QG_CA_Contig5046	1	2.4	aggattcgcgactgaatttg	agtgctgcttcccagtcttc
LK1151*	QG_CA_Contig1246	1	38.0	ccaagttcttgagcctccac	tctgcaggagcgatttcac
NL0311		1	48.4	gtcttggagcaacaccttc	aatgccacttaagctttcac
NL1324*		1	59.1	cataaccggaagcttgttc	tgcataaagaaatatgcaaaac
NL0460		1	64.1	cgattttcatacactctgcc	ttgattgcctctgtgtttg
NL0267		1	73.5	ggcagtgggtgtaaatgac	tgactggatcagcagattg
QGF24O17	QGF24O17.yg.ab1	1	85.4	gtttctccccctttcagctt	tcccaaacatggtggatctt
QG_CA6637	QG_CA_Contig6637	1	87.6	cattgtgctcgttgcagagt	gcaccaacctcgttcaattt
SCW09	Paran & Michelmore[1]	2	-5.3	gtgaccgagtagtcttaacctagt	gtgaccgagtgtaacaacgtaaat
SCV12	Paran & Michelmore[1]	2	-4.0	acccccactaccatatcaatctc	acccccacttgtcctgcaacttt
LK210	QG_CA_Contig4663	2	0.0	attccatccaccgatgttgt	aaattggcaagcatctcagc
NL1283		2	3.8	atcgggttttgtgattttg	aatttcgacgaaccaacac
NL0736		2	24.8	gagaaatgtccgaaactgc	ctcaagtcctttgcctgac
NL0593		2	31.9	gaagcagaagttgtgaggc	caaagcacttggatccttc
NL0967		2	42.2	aagaagtgacattccggtg	ttccacattcgtcaagattc
LE1276	QG_CA_Contig7689	2	60.2	tttgggttccttcagtttgc	cacagtttgggatgaacacg
LK1475	QG_CA_Contig7099	2	74.4	ggagttcagggcctctgtc	ccgattctgcggttatcttc
NL0842		2	86.2	ttctatccgtttgggaatg	tgctgctgatttaccacac
NL0319		2	92.3	gctgactggatttaggacg	gtctgactgtccattttgttg
NL0561		3	4.4	tacagtcgacgtttcttgc	gggtaaagacggagaaacc
QGA8B05	QGA8B05.yg.ab1	3	14.2	tggatgatgtgccgataaga	cctgttgccttgaaattggt
QG_CA1077	QG_CA1077	3	32.1	tagggcctcctcttccattt	aaacagcttcggcttcaaaa
NL1124		3	41.4	ccgttactttagtccgctg	cttccacctttttgagacg
NL1049		3	52.5	gccatttaacagatttgtgtg	ctgaagtgtctttttattaccac
NL1174		3	68.5	gagcatctgatctccgttc	tgattggaattgggcttac
NL0117		3	80.8	gtacaatggagatggtggg	tctgatctgaaatcccgac
NL1151		4	4.5	tttggtatcaagcctctcg	caagtctatcaagccctgg
NL1260*		4	8.2	cttagaaagctgccaccac	ggagcgattttacagttcg
NL0897		4	18.4	gaagacaagaagtcgacgg	cgatcgagataacgaaagc
NL1088		4	43.3	atttgaaagccatggaaac	ttgcttcaaattttccacc
LK1527	QG_CA_Contig7732	4	48.9	aagttcttccgatcccttcc	ccctgtttcctcctttaccc
NL0531		4	59.9	gtcggtatcaaattaggcg	aggcagagattggatgatg
NL0207		4	75.0	atatccgtcattgtcgtcg	caaagcccatatgaaaaatg
NL1337		4	87.9	cttcgtggaaggttttcag	atcttgtgccatggtaagc
LE1233	QG_CA_Contig5723	4	102.0	caggattcttcaggagcagc	cccaatctcgtccactgttt
LE1162	QG_CA_Contig345	4	136.5	taaagaggatctcatgggcg	gaatgcaacatatgcaaccg
NL1035		4	154.2	atgcaatagaccttggtgc	ttgtccacctcccaaatac
LE0354	CLX_S3_Contig7850	5	-6.0	ggatgcggttaaagaagcaa	ccccattaaacggaattgtg
NL1090		5	3.0	actcaatgcacgattctcc	tcagtgtaagtgcctgtgg
NL0103*		5	25.5	acacaaatcaagggaatgc	tccctgactgagtggagtc
NL0853		5	45.2	ttcctgtgtttgtgtcagg	cggaattacaaccaacattac
NL0173		5	59.0	cgcagagatagagacaggg	acgtgcaataaaccaaacc
NL0871		5	76.4	tttattcatgggtcaagcc	gaaccgaaggatttcacac
NL1220		5	80.4	tcccacagtttcctcattc	aaatcgccatttacgactg
NL1159		5	94.5	caaatcgtacttccgcttc	acacctggagattttgtgc
NL0750		5	100.8	tgtgtattttatgcgcacc	ttgctctcactgatctccc
NL0889		5	120.5	gtcgccatatcaaaagagg	gagcaaacatgcaaatagg

Marker name	Contig or publication	Chr.	Position (cM)	Primer foreward	Primer reverse
LK0205	QGA13L14.yg.ab1	6	6.1	gtgtgatcctgcatcccaat	tagtcgcgccctgtttacat
LK1471	QG_CA_Contig6524	6	14.3	tggcatggaatttgaatcag	ccttgacactcctcccattc
CLX_S3_14099	CLX_S3_Contig14099	6	28.2	agaagcaagctccatgagga	tagtcggaaaacgccagttc
CLS_S3_8361	CLS_S3_Contig8361	6	34.4	tctgcaaccatctgaagcac	cccatttgcaccatacacag
NL1117		6	50.0	actgtctccaccgaagatg	ttggttacagggattttgg
LE0178	QGG26M07.yg.ab1	6	57.7	ttgctgacataagagaagtttcaa	gtatcatccacatcgtctaga
LE1126	QG_CA_Contig1905	6	66.3	ctttgctccaattcctctcg	aatgccatagtgaagctggg
LE1211	QG_CA_Contig4578	6	81.9	cgggtgattacatcggctat	cgcaaccaaccaaatttacc
LK1504	QG_CA_Contig1477	7	0.0	gcatcaggaaatccgagtgt	ccgcctagggttcttcctac
NL1034		7	33.6	gaacaggaacaaaacccag	acctgtgtgggtctcaaag
NL0650		7	47.0	gggaaacgtaatagaacgg	aatctcgtggcaaatatgg
LK1513	QGF20P01.yg.ab1	7	54.9	cgaagacaaagcctggaaag	ttgcagtacacagcaacacg
LE0190	QG_CA_Contig1870	7	69.9	cgtccctactgtgagcaaca	atacgagagaccgcgtgcta
QGG16B23	QGG16B23.yg.ab1	8	0.0	agcctcccacatcatttgaa	aaagcccagcaactaccaca
LE1065	QG_CA_Contig2688	8	11.6	gtgaaaaccagccctaccaa	aggcttcccaacattcacac
CLS_S3_9019	CLS_S3_Contig9019	8	18.0	tctaccatgggcaagaccac	ccattcagaagtcgctccag
NL0935		8	21.9	gtgaaccaatgagtggagg	gaacatccacttggtccag
KLE0263	QGH6L10.yg.ab1	8	30.2	caacctcaccggagttttgt	gccggaaagtttgttgttgt
KLK1366	QGI7O15.yg.ab1	8	37.7	gaatcgctcaggcaaacaat	tggcctctcaagcagatttt
LE3019	QG_CA_Contig2149	8	51.4	attgctggagtcgtggtttc	ctttgtgcctcaaacccaat
NL0455		8	76.0	gacaagctcaaggcaactc	tgatcatctacatagcttcactg
CLS_S3_6304	CLS_S3_Contig6304	8	99.0	ctgtatgtggtccggcaagt	tcatcccgccataaccataa
NL0159		8	107.3	atgtgtaaccagtcggagg	cctgaacgcaataacttcc
RIN4	Jeuken et al, 2009	9	6.7	cgagcagggaagagaatgag	tagagggagtcccatggcta
M431	QG_CA_Contig6010	9	20.0	gatcgatcgttcatcgttctctca	ttgttgaaacaagttcactatttgg
CLX_S3_6726	CLX_S3_Contig6726	9	31.1	ggacgatggttttggagcta	acgagcagcttcacgatttt
NL1302		9	42.8	tttccagatgaaatccctg	atcaatggcttcctgtgtc
KM2348	QG_CA_Contig2348	9	65.6	taaacttcgggacgaaccac	gccaaaatgcgaaagttgc
CLX_S3_8409	CLX_S3_Contig8409	9	70.0	tcccgataaagaccctgatg	aggaggaactgaacgatgga
CLX_S3_8498	CLX_S3_Contig8498	9	89.6	ggataggaggaggtgggaag	ggtcaccggctaatacctca
CLX_S3_12996	CLX_S3_Contig12996	9	95.1	tcttggcctctcattgatcc	ccaacggggaacacaaatac
CLSX3110_K09_2		9	106		
CLS3349		9	107.0		
CLS4696_2	CLS_S3_Contig4696	9	100.0	aatctccagcttcgggtttt	actacgaaacgacccattgc
CLS_S3_4656	CLS_S3_Contig4656	9	110.4	ccgtatgccgttcatcttct	gcactccaattgaatgatcg

[1] I. Paran and R. W. Michelmore, 1993.

*These markers failed to be informative. Reason for failure is unknown.

All markers starting with NL are SSR markers, other markers are EST based markers.

This Table is copied from (den Boer et al. 2014), markers which are not used in this Thesis are deleted form this table.

Apendix 2 All genotypic data for mapping with BC1_GEO

All genotypic data is presented here below per marker per plant (nrs in the first row, nr 5 and 36 were F_2-plants and therefore excluded from this data set).

Genotypic codes: a= homozygote *L. sativa* cv. Olof; h= heterozygote; u=unknown

	cM	marker	1	2	3	4	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65
C1	2,4	LK1549	a	h	a	a	h	a	a	h	h	h	a	h	h	h	a	h	a	a	h	h	a	u	h	h	a	h	h	a	h	a	h	h	h	a	h	h	h	a	a	h	h	h	h	h	h	a	h	h	a	h	h	a	a	h	h	a	h	a	a	a	a	h	a
C1	48,4	NL0311_WUR	a	a	a	a	h	a	u	a	h	h	u	h	h	a	u	h	a	a	h	h	h	a	u	h	u	h	a	u	a	h	u	h	h	a	h	u	h	a	h	h	h	h	u	a	u	a	a	a	a	a	u	a	h	a	a	a	h	a	h	a	a	a	h
C1	64,1	NL0460	h	a	a	a	h	a	h	a	h	a	a	h	h	h	h	h	h	a	h	h	h	h	h	h	a	h	a	h	a	h	h	h	h	h	h	a	h	h	h	h	a	h	a	a	a	a	a	a	a	a	h	a	u	a	h	a	h	h	h	h	a	u	h
C1	73,49	NL0267	h	a	a	a	h	a	h	h	h	a	h	h	h	h	h	h	h	u	h	h	h	h	h	h	a	h	a	h	a	h	h	h	h	h	a	a	h	h	h	h	a	h	a	a	a	a	a	a	a	a	h	a	a	a	h	h	h	a	h	h	h	a	h
C1	85,4	QGF24O17	h	a	a	h	h	a	u	h	h	a	h	h	h	h	h	h	h	a	h	h	h	h	h	h	a	u	u	h	a	h	h	h	u	h	a	a	h	h	h	h	a	a	a	a	a	a	a	a	a	a	h	u	a	a	h	h	h	a	h	h	h	h	u
C1	87,6	QG_CA6637	h	a	a	h	h	a	h	h	h	a	h	h	h	h	h	h	h	a	h	h	h	h	h	h	a	h	a	h	a	h	h	h	h	h	a	a	h	h	h	h	u	a	a	a	a	a	a	a	a	a	h	a	a	a	h	h	h	a	h	h	h	h	h
C2	-4,0	SCV12	a	h	h	a	a	h	a	h	h	a	h	a	a	h	h	a	a	a	a	a	h	h	a	a	a	h	a	a	h	a	a	h	h	h	h	a	h	a	a	h	a	h	a	h	a	a	h	h	a	h	h	h	a	h	h	a	a	h	h	a	a	a	h
C2	0,0	LK210	a	a	h	a	a	h	a	h	h	a	h	a	h	h	h	a	a	a	a	a	h	h	a	u	a	a	a	a	h	a	a	u	h	h	h	a	h	u	a	h	a	h	a	h	a	u	h	h	a	h	a	h	h	u	h	a	a	a	h	a	a	u	u
C2	3,8	NL1283	a	a	h	a	a	h	a	h	h	a	h	a	h	h	h	a	a	a	a	a	h	h	a	a	a	a	a	a	h	a	a	h	h	h	h	a	h	a	a	h	a	h	a	h	a	a	h	h	a	h	a	h	h	h	h	a	a	a	h	a	a	a	h
C2	24,7	NL0736	a	a	h	a	a	a	a	h	h	h	h	a	h	a	a	a	a	a	a	a	a	h	a	a	a	a	h	h	h	a	a	h	h	h	a	h	h	a	a	h	a	h	a	a	a	a	h	a	h	h	a	a	h	h	h	h	a	a	h	a	h	a	h
C2	31,9	NL0593	a	a	h	a	h	a	a	h	h	h	h	a	h	a	a	a	a	a	a	h	a	h	a	a	a	a	h	a	h	a	a	h	h	h	a	h	h	a	a	h	a	h	a	a	a	a	a	a	h	h	a	a	h	h	h	h	a	a	h	a	h	a	h
C2	42,2	NL0967	a	a	a	h	h	a	a	a	h	h	h	a	h	a	a	a	a	a	a	h	a	h	a	a	a	a	h	a	h	a	a	h	h	h	a	h	h	a	a	a	a	h	a	a	a	a	u	a	h	a	a	a	h	h	h	h	a	a	h	h	h	a	h
C2	60,2	LE1276	a	h	a	h	h	h	a	a	u	h	h	a	h	h	a	h	h	a	a	h	a	a	a	h	h	a	h	a	h	h	h	h	h	h	u	h	u	a	a	a	a	h	h	h	a	a	a	a	h	u	a	a	h	h	h	a	h	a	h	h	h	a	h
C2	74,4	LK1475	a	h	a	h	h	h	a	a	a	h	h	a	h	h	a	h	h	a	a	h	a	a	h	h	h	a	h	a	h	h	h	h	h	h	h	a	h	a	a	a	a	h	h	h	a	h	a	a	h	a	a	a	h	a	h	a	h	h	h	a	h	a	h
C2	81,9	NL0899	a	h	a	h	h	h	a	a	a	h	h	a	h	h	a	h	h	a	a	h	a	a	h	h	h	a	a	a	h	h	h	h	h	h	h	a	h	a	a	a	a	h	h	h	a	h	a	a	h	a	a	a	h	a	a	a	h	h	h	a	h	u	h
C2	86,2	NL0842	a	h	a	h	h	h	h	a	a	h	u	a	h	h	h	h	h	a	a	a	a	a	h	h	h	a	a	a	h	h	h	h	h	h	u	u	h	a	a	u	u	h	h	h	a	h	a	a	h	a	a	a	h	a	a	a	h	h	h	a	h	a	h
C2	92,3	NL0319	a	h	a	h	h	h	h	a	a	h	h	a	h	h	h	h	h	a	a	a	a	a	h	h	h	a	a	a	h	h	h	h	h	h	h	a	h	a	a	a	a	h	h	h	a	h	a	a	h	a	a	a	h	a	a	a	h	u	h	a	h	a	h
C3	4,4	NL0561	h	a	a	h	a	h	h	a	h	h	a	h	a	a	a	a	h	a	a	h	a	a	a	h	h	a	h	h	h	a	a	u	a	a	u	h	h	h	h	h	a	h	h	a	a	h	h	a	a	h	a	h	a	h	a	a	a	a	h	h	a	h	h
C3	14,2	QGA8B05	h	h	a	h	a	h	h	h	h	h	a	h	a	a	a	a	h	a	a	h	a	h	a	h	h	a	h	h	h	a	h	h	h	a	h	h	h	h	h	h	a	h	h	a	a	h	h	a	a	h	a	h	a	h	u	a	a	a	h	h	a	h	u
C3	32,1	QG_CA1077	h	h	a	h	a	h	h	h	h	h	a	h	a	a	a	h	h	a	h	a	a	h	a	h	a	a	u	h	h	h	h	a	h	a	h	h	h	h	a	h	a	h	h	a	a	h	h	a	a	h	a	h	a	h	a	a	h	a	h	h	a	h	h
C3	41,4	NL1124	a	h	a	h	a	h	a	h	a	h	h	a	a	a	a	h	h	a	h	a	a	h	a	h	a	h	h	h	h	h	h	a	h	a	h	u	h	h	a	h	a	h	h	a	a	a	h	a	a	h	a	h	a	h	a	a	h	a	h	h	a	h	h
C3	52,5	NL1049	a	h	a	a	a	h	a	h	a	h	h	a	a	a	a	h	h	a	h	a	a	h	a	h	a	h	h	h	h	h	h	a	h	a	h	a	h	h	a	h	a	a	h	a	a	a	h	a	a	h	a	h	a	a	a	a	h	h	h	h	h	h	a
C3	68,5	NL1174	a	h	a	a	a	a	a	h	a	h	h	a	h	a	a	h	h	h	a	a	h	h	a	h	a	h	h	a	h	h	h	a	h	h	h	a	h	h	h	h	a	a	h	a	a	a	a	a	h	h	h	a	a	a	a	a	h	h	a	h	h	h	a
C3	80,8	NL0117	a	h	a	h	a	a	a	h	a	h	h	a	h	a	a	h	h	a	a	a	a	h	a	h	a	h	h	a	h	h	h	a	h	a	h	a	h	a	h	h	a	h	h	a	a	a	a	a	h	h	h	a	a	a	a	a	h	h	a	h	h	h	a
C4	4,5	NL1151	a	h	h	h	h	a	h	h	h	a	h	h	h	h	h	a	a	h	h	a	a	h	h	h	h	a	a	a	h	h	a	h	h	h	h	h	a	h	a	h	h	h	h	h	h	h	h	h	a	h	h	h	a	h	h	a	h	h	a	a	a	a	h
C4	18,4	NL0897	a	h	a	h	h	a	a	h	h	h	h	a	h	h	h	a	a	h	a	a	a	a	h	h	a	a	a	a	h	h	h	h	a	h	h	h	h	h	a	h	h	a	h	h	h	h	a	h	a	h	a	h	a	h	h	a	h	a	a	a	h	h	h
C4	43,3	NL1088	a	h	a	a	h	a	a	h	h	h	h	a	h	h	h	a	a	h	a	a	a	a	h	h	a	a	a	h	h	h	h	h	a	h	a	a	h	h	h	h	h	a	h	h	h	a	a	h	a	h	a	h	a	h	h	h	h	a	h	a	h	h	h
C4	48,9	LK1527	a	h	a	a	h	a	a	h	h	h	h	a	h	h	h	a	a	h	a	a	a	a	h	h	a	a	a	h	h	h	h	h	a	h	a	a	h	h	h	h	h	a	h	h	h	a	a	h	a	h	a	h	a	h	h	h	a	a	h	a	h	h	h
C4	59,9	NL0531	a	h	a	a	h	a	a	h	h	h	h	a	h	a	h	a	a	h	a	h	a	a	a	h	a	a	a	h	h	h	h	h	a	h	a	a	h	h	h	h	h	a	a	h	h	a	a	h	a	h	a	h	a	h	h	h	a	a	h	a	h	h	h
C4	75,0	NL0207	a	a	a	h	h	a	a	h	h	h	h	h	h	a	h	a	a	h	a	h	a	a	a	h	h	a	a	h	h	h	a	h	a	h	a	a	h	h	h	h	h	a	a	a	h	a	a	h	h	h	a	a	a	h	a	h	a	a	h	a	a	h	h
C4	87,9	NL1337	a	a	u	h	h	a	h	h	h	a	h	h	h	a	h	a	a	h	a	h	h	h	a	h	h	a	a	h	h	h	a	h	a	a	a	a	h	h	h	h	a	a	a	a	h	h	a	h	h	h	a	a	a	h	a	h	a	a	h	a	a	h	h
C4	102,0	LE1233	h	a	h	h	h	h	a	a	h	a	a	a	h	a	a	h	a	h	h	h	h	h	h	h	h	h	a	h	a	a	h	h	a	a	a	a	h	h	h	a	a	a	a	a	h	h	h	a	h	h	a	a	a	h	a	h	h	a	h	h	h	h	h
C4	136,5	LE1162	h	a	a	h	h	h	a	a	h	a	a	a	a	h	h	h	a	u	h	a	h	h	h	h	h	h	a	a	a	a	h	h	a	h	a	a	a	h	h	u	a	h	u	a	h	h	h	u	h	h	a	a	h	h	a	a	h	a	h	h	h	h	a
C4	154,2	NL1035	a	a	a	h	h	h	a	a	a	u	a	a	a	h	h	h	a	h	h	a	h	h	h	h	a	h	h	a	a	h	h	h	a	h	h	a	a	h	h	a	h	h	h	a	h	h	h	a	h	h	a	a	h	h	a	a	a	a	h	h	h	a	a
C5	-6,0	LE0354_2	a	h	a	a	a	a	h	a	a	a	h	h	h	a	a	h	a	a	h	a	h	a	a	a	a	h	a	h	h	h	h	u	h	a	h	h	h	a	a	h	h	a	h	a	h	a	h	a	a	a	h	u	a	a	a	a	h	a	h	a	h	a	h
C5	3,0	NL1090	a	h	a	a	a	a	h	a	a	a	h	h	h	a	a	h	a	a	h	a	h	a	a	a	a	h	a	h	h	h	h	a	h	a	h	h	h	a	a	h	h	a	h	a	h	a	h	a	a	a	h	h	a	a	a	a	h	a	h	a	h	a	h
C5	45,2	NL0853	a	h	a	a	a	h	a	a	h	h	a	h	h	h	a	h	h	a	a	a	h	a	a	a	a	h	a	h	a	h	h	a	h	a	a	a	h	a	a	h	h	h	h	a	h	h	h	a	h	a	h	h	a	a	a	h	u	h	h	a	a	a	h
C5	59,0	NL0173	a	h	a	a	h	h	h	a	h	a	a	h	a	a	h	h	h	a	a	a	a	a	a	a	a	h	a	h	h	h	h	h	a	a	a	a	h	a	h	h	h	h	h	a	h	h	h	a	h	h	h	h	h	h	a	a	a	h	h	h	h	a	h
C5	76,4	NL0871	a	h	a	a	h	h	h	a	h	u	a	h	a	a	h	h	h	u	a	a	a	h	h	a	h	a	a	h	h	a	h	h	a	u	h	a	h	a	h	h	u	h	h	a	h	h	h	h	h	h	h	h	h	h	u	a	a	h	h	h	h	u	h
C5	80,4	NL1220	a	a	a	a	h	h	h	a	h	a	a	h	a	a	h	h	h	a	a	a	a	h	h	a	h	a	a	h	h	a	h	h	a	a	h	a	h	a	h	h	h	h	h	a	a	h	h	a	h	h	h	h	h	h	a	a	a	h	h	h	h	a	h
C5	94,5	NL1159	a	a	a	a	h	h	u	a	h	a	a	a	a	a	h	h	h	h	a	h	a	h	h	a	h	a	a	h	h	a	h	h	a	a	h	a	h	a	h	a	h	h	h	a	a	a	h	a	h	h	h	h	h	a	a	h	a	h	h	h	h	a	h
C5	100,8	NL0750	a	a	a	a	u	u	u	a	u	a	a	a	a	a	h	h	u	u	a	h	h	h	h	a	h	a	a	h	u	a	h	h	a	a	h	a	u	a	h	a	u	h	h	a	u	a	a	a	u	h	h	h	h	a	a	h	a	h	h	h	h	a	u
C5	120,5	NL0889	a	a	a	a	h	h	a	a	h	a	a	a	a	a	h	h	h	h	h	h	h	a	h	a	h	h	a	h	u	a	h	a	a	h	h	a	h	a	h	a	a	h	h	u	h	a	a	a	h	a	h	h	h	a	a	h	a	h	h	h	h	h	h

	cM	marker	1	2	3	4	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65
C6	6,1	LK0205	a	a	h	a	a	u	h	a	h	h	h	h	a	a	h	h	a	h	a	h	a	h	a	a	a	a	a	a	h	a	h	h	h	a	h	a	a	a	a	a	a	a	h	h	a	h	a	h	h	u	h	a	h	a	a	a	h	h	h	h	h	h	a
C6	14,3	LK1471	a	a	h	a	a	h	h	a	h	h	h	h	a	a	h	h	a	h	a	h	a	h	a	a	a	u	a	a	h	a	h	h	h	a	h	a	a	a	a	a	a	u	h	h	a	h	a	h	h	h	h	a	h	u	a	a	h	h	h	h	h	h	a
C6	28,2	CLX_S3_14099	a	a	h	a	h	h	a	a	h	h	h	h	a	a	h	h	a	a	a	h	h	h	a	a	a	a	h	a	a	a	h	h	h	a	h	h	a	a	a	a	a	a	h	h	a	h	h	h	a	h	a	a	a	a	h	a	h	h	h	a
C6	34,4	CLS_S3_Contig83	a	h	h	a	u	h	a	a	h	h	h	h	a	a	h	h	a	a	a	h	h	h	a	a	a	a	h	h	a	a	h	h	h	a	h	h	a	a	a	a	a	a	h	h	a	h	h	h	a	h	a	a	a	a	h	a	h	h	h	a
C6	50,0	NL1117	h	h	h	a	h	a	a	h	h	a	a	h	a	a	h	h	a	a	h	h	h	h	a	a	a	a	h	h	a	h	a	h	a	a	h	a	h	a	a	a	h	h	h	a	h	h	a	a	h	h	h	h	h	a	h	a	a	a	h	h	h	h	h
C6	57,7	LE0178	h	h	h	a	a	a	a	a	h	a	a	a	a	a	a	h	a	a	h	h	h	h	h	a	a	a	h	h	a	h	a	h	a	a	h	h	a	h	a	a	a	u	h	h	a	h	h	a	a	u	u	h	h	h	a	h	a	h	a	h	h	h	h
C6	66,3	LE01126	h	h	h	h	a	a	h	a	h	a	h	a	h	a	h	a	a	h	h	h	h	h	h	a	a	a	h	h	h	h	h	h	h	a	h	h	a	h	a	a	a	a	h	h	h	a	h	h	a	a	a	a	a	a	h	a	h	h	h	a
C6	81,9	LE1211	h	h	h	h	a	a	h	a	h	a	h	a	h	h	a	h	a	h	h	a	h	h	h	a	h	a	a	h	h	h	h	h	a	a	h	a	a	h	a	h	a	a	h	h	h	a	h	h	a	h	a	a	a	a	a	h	a	h	a	a	a	u	a	h	h	a
C7	0,0	LK1504	a	a	h	h	a	h	a	h	h	h	a	h	a	h	a	h	h	h	h	a	h	h	a	a	a	a	a	a	a	h	h	a	h	h	h	u	h	h	h	h	h	a	a	h	h	a	a	a	h	a	a	u	h	h	a	a	a	a	a	h	h
C7	33,6	NL1034	a	a	h	h	a	h	a	h	h	h	a	a	a	h	a	a	a	a	h	a	a	a	a	a	a	a	a	h	h	a	a	h	h	h	a	h	h	h	h	h	a	h	a	a	h	h	a	a	h	a	a	a	h	h	a	h	h	h	h	h	h	h	h
C7	47,0	NL0650	a	a	h	h	a	h	a	h	h	h	h	a	h	h	a	a	a	a	a	h	a	a	h	h	a	h	h	h	u	h	a	a	h	h	h	a	h	a	h	h	h	a	a	a	a	a	h	a	a	h	h	h	a	a	h	a	a	a	h	h	a	h	h	h
C7	54,9	LK1513	a	a	h	h	a	u	a	h	u	u	h	h	u	h	a	a	a	a	h	a	a	u	h	a	u	h	u	a	u	a	a	h	h	h	a	u	a	h	h	u	a	a	a	a	a	u	a	a	h	h	u	a	a	h	a	a	a	h	h	a	h	h	h
C7	69,9	NL0851	a	a	h	h	u	a	h	h	h	h	h	h	h	h	a	a	a	a	h	a	a	h	h	a	h	h	h	a	h	a	h	a	h	h	a	h	u	h	h	h	a	a	a	a	u	a	a	u	h	h	h	u	a	h	a	a	a	h	h	a	h	a	h
C8	0	QGG16B23	h	h	a	h	h	a	a	h	h	a	a	h	h	h	a	h	h	h	h	h	a	a	a	a	a	h	a	h	h	h	a	h	h	h	a	h	h	h	h	a	h	h	h	a	a	h	a	a	a	h	h	h	h	a	h	h	a	a	u
C8	11,6	LE1065	h	h	a	h	h	h	a	h	h	a	a	h	h	h	a	h	h	h	h	h	h	h	a	a	a	h	a	h	h	h	a	h	h	a	h	h	u	h	h	h	h	u	h	a	h	h	h	a	h	h	a	a	h	h	h	h	a	h	h	a	a	h
C8	18,0	CLS_S3_9019	h	h	a	h	h	h	a	h	a	a	a	h	h	h	a	a	h	h	h	h	h	a	a	a	a	h	h	h	h	h	a	h	h	a	h	h	h	h	h	h	h	h	a	h	h	a	a	h	h	h	a	a	h	h	h	h	u	h	h	a	a	u
C8	21,9	NL0935	h	h	a	h	h	h	a	h	u	a	u	h	h	h	a	a	h	u	h	h	h	a	a	a	u	h	h	h	h	h	a	h	h	a	h	h	h	h	h	h	h	h	a	h	h	a	u	u	h	u	h	a	a	h	h	h	a	h	h	a	a	h
C8	30,2	KLE0263	a	h	a	h	h	h	a	h	a	a	a	h	h	h	a	a	h	h	h	h	h	a	a	a	a	h	h	h	h	h	a	h	h	h	h	h	h	h	u	h	h	h	h	a	a	h	h	a	a	h	a	a	h	h	h	a	a	h	h	h	u	h	h	h	a	a
C8	37,7	KLK1366	a	h	a	h	h	h	a	h	a	a	a	h	h	h	a	a	h	h	h	h	h	u	a	a	a	h	h	h	h	h	a	h	h	h	h	h	h	h	h	h	a	a	h	h	a	a	h	a	a	h	h	h	a	a	h	h	h	a	a	h	h	a	h	h	a	a
C8	51,4	LE3019	a	h	a	h	h	h	u	h	a	a	a	h	h	h	a	a	h	h	a	h	a	a	h	a	a	h	h	h	h	h	h	h	h	h	h	h	a	h	h	h	h	h	a	a	a	h	h	a	a	h	a	a	h	h	a	a	a	h	h	a	a	a	h	h	a	a
C8	76,0	NL0455	a	h	a	h	a	a	a	h	a	h	a	h	h	h	a	a	h	h	a	h	a	a	h	a	a	h	h	h	h	h	h	h	h	h	a	h	h	h	h	a	h	a	h	h	h	a	a	a	h	h	h	a	a	h	h	a	a	h	h	a	a	h	a	a	u
C8	99,0	CLS_S3_6304	a	h	a	h	a	u	h	h	a	h	a	h	h	h	a	a	u	a	a	h	a	a	h	a	a	h	h	u	h	h	h	h	h	h	h	h	a	h	a	h	h	h	u	a	a	a	h	h	a	a	h	h	a	a	h	h	a	h	h	a	h	a	a	a
C8	107,3	NL0159	a	h	a	h	a	a	h	h	a	h	a	h	h	h	a	a	h	a	a	a	a	a	h	a	a	a	h	a	h	h	h	h	h	h	h	a	h	a	h	h	h	a	a	a	a	h	a	h	h	a	h	a	a	a	h	h	a	h	h	a	h	a	a	a
C9	6,7	RIN4_Snip_4	a	a	a	h	a	h	h	a	a	a	h	h	h	h	a	a	h	h	h	h	h	h	a	a	h	a	h	a	h	h	a	h	a	a	h	h	a	h	a	a	a	a	h	a	a	a	h	h	a	h	a	h	a	h	a	h	h	h	h	a	h	a	h	a
C9	20,0	M431dCAPs	a	u	a	h	u	h	a	a	a	a	a	h	u	h	a	u	u	h	h	h	h	h	a	a	h	h	h	a	h	u	u	h	a	u	u	h	u	h	a	h	a	a	h	a	a	a	h	h	a	h	a	h	a	h	u	u	h	h	h	h	a	h	u	h	a
C9	31,1	CLX6726	a	a	a	h	a	h	u	a	a	a	a	h	h	h	a	a	h	h	h	h	a	h	a	a	h	h	h	a	a	a	a	h	a	a	h	h	a	h	h	h	a	h	a	a	a	a	a	h	h	a	h	a	h	a	a	a	u	h	h	h	a	a	a	h	u	a	a
C9	42,8	NL1302	h	a	a	h	a	h	u	h	a	a	a	h	h	h	a	a	h	h	h	h	a	h	h	a	h	h	h	a	a	a	a	h	a	a	h	h	a	h	a	h	h	h	h	h	a	h	a	h	h	a	h	a	h	a	a	a	h	a	a	u
C9	65,6	KM2348	h	a	a	h	a	h	a	h	a	a	a	h	h	u	a	a	a	h	a	h	a	h	h	a	a	h	h	a	a	a	a	h	a	a	a	h	a	h	a	h	h	h	h	h	a	a	h	a	a	a	h	a	a	a	h	a	a	a	h	a	a	a
C9	70,0	CLX8409	h	a	a	h	a	h	a	h	a	a	a	h	h	h	a	a	a	h	a	h	a	a	h	a	a	h	h	a	a	a	a	h	a	a	a	h	a	h	h	h	h	h	a	a	h	h	h	h	h	h	a	h	a	a	a	h	a	a	a	h	a	a	a	a
C9	95,1	CLX12996	a	h	a	h	h	a	a	h	a	a	a	a	h	h	a	a	u	u	a	a	a	a	a	a	a	h	a	a	h	a	a	a	u	a	a	a	a	a	a	h	h	a	a	a	a	h	a	a	a	a	a	a	h	a	a	a	h	a	a	u
C9	100,0	CLS4696_2	a	h	a	h	a	a	a	a	a	a	a	a	h	h	a	a	a	a	a	a	a	a	a	a	a	h	a	a	h	a	a	a	a	a	a	a	a	a	a	a	h	a	a	a	a	a	a	h	a	u	a	a	a	a	a	a	h	a	a	a
C9	106	CLSX3110	a	h	a	h	a	a	a	a	a	a	a	a	h	h	a	a	a	a	a	a	a	a	a	a	a	h	a	a	h	a	h	a	u	a	a	a	u	a	a	h	a	a	u	u	a	u	u	u	u	u	u	h	a	a	a	a	a	a	h	a	a	a
C9	107	CLS3349	a	h	a	h	a	a	a	a	a	a	a	a	h	h	a	a	a	a	a	a	a	a	a	a	a	h	a	a	h	a	h	a	a	a	a	a	a	a	a	a	h	a	a	a	a	a	a	a	a	a	h	a	h	a	a	a	a	a	a	h	a	a	a
C9	110,4	CLS_S3_4656	a	h	a	h	a	a	a	a	a	a	a	a	h	h	u	a	a	a	a	a	a	a	a	a	a	h	a	a	h	a	h	a	a	a	a	a	a	a	a	u	a	a	a	a	a	a	a	a	a	a	h	a	h	a	a	a	a	a	a	h	a	a	a

Apendix 3 All phenotypic data for mapping with BC1_GEO

Plant numbers in the first column.

	ADTg1	ADTg2	ADTg3	Bolting (the central stem in cm above the soil)			The presence of sideshoots (1=yes 0=no)			Shade of green 0=light 1=in between 2=dark	The presence of a lot of anthocyan 1=yes 0=no	Leaftip shape 0=pointy 1=pointy (bit round) 2= in between 3=round (bit pointy) 4=round	Twisted leafs 0=none 1=a few 2=around half 3=almost all
	11dpi	**9dpi**	**9dpi**	**46dps**	**55dps**	**63dps**	**46dps**	**55dps**	**63dps**	**63dps**	**63dps**	**63dps**	**63dps**
1	80.0	82.5	57.5	5	6	8	0	0	0	0	0	4	0
2	3.0	23.7	0.5	5	11	14	0	0	1	2	0	0	3
3	8.0	45.0	50.0	8	41	41	0	1	1	0	0	4	0
4	7.2	11.0	0.0	19	41	41	1	1	1	0	0	0	0
6	51.3	65.3	78.8	4	4	5	0	0	1	2	0	4	0
7	85.0	41.8	5.7	14	40	41	1	1	1	2	0	0	0
8	83.8	88.8	95.0	4	5	8	0	0	0	0	0	4	0
9	47.5	53.3	85.0	4	7	7	1	1	1	2	0	0	0
10	31.3	23.3	62.5	5	7	9	0	1	1	2	0	4	0
11	12.8	32.5	61.3	6	20	41	0	1	1	2	0	0	2
12	50.0	80.0	63.0	10	41	41	1	1	1	2	0	0	1
13	71.2	57.5	12.8	8	32	41	1	1	1	0	0	0	0
14	22.5	26.5	0.3	12	41	41	1	1	1	2	0	0	2
15	0.8	0.3	0.0	11	40	41	1	1	1	0	0	0	0
16	48.8	56.3	73.8	5	7	8	0	0	0	2	0	4	0
17	62.5	87.5	14.8	3	5	5	0	1	0	2	0	2	0
18	82.5	81.3	43.0	4	4	7	0	0	1	2	0	4	0
19	3.2	24.7	10.8	4	4	4	0	0	0	2	1	4	0
20	73.7	38.7	0.3	9	31	41	1	1	1	0	0	0	0
21	66.3	63.8	48.3	4	4	5	0	0	0	2	0	4	0
22	50.5	81.3	45.0	4	5	7	0	0	0	0	0	1	0
23	34.2	7.3	2.2	8	29	41	1	1	1	0	0	1	0
24	37.8	34.3	98.8	4	15	41	0	0	1	2	0	3	0
25	13.0	66.5	71.3	4	5	7	0	0	0	2	0	3	0

26	47.5	47.5	67.5	5	38	41	1	1	1	0	0	4	0
27	1.0	3.7	0.3	9	39	41	1	1	1	2	0	0	0
28	75.0	41.5	10.0	14	41	41	1	1	1	2	0	2	0
29	26.5	52.5	18.5	4	5	7	0	0	0	2	0	4	0
30	1.5	6.3	0.0	4	10	29	0	1	1	2	0	4	2
31	22.8	72.5	32.3	4	4	7	0	0	1	2	0	0	3
32	0.0	0.0	1.2	5	36	41	1	1	1	2	0	0	0
33	31.7	10.8	2.0	8	11	17	1	1	1	2	0	3	0
34	24.2	17.5	1.5	10	40	41	1	1	1	0	0	0	0
35	0.0	0.0	0.0	4	8	21	0	0	0	2	0	4	0
37	81.3	83.8	41.3	4	4	5	0	0	0	2	0	4	0
38	71.3	17.3	0.0	21	41	41	1	1	1	0	0	4	0
39	33.8	65.0	25.3	3	3	5	0	0	0	2	0	4	0
40	75.0	31.3	0.0	12	41	41	1	1	1	2	0	3	0
41	33.0	0.2	0.0	14	41	41	1	1	1	2	0	0	0
42	5.0	6.2	0.0	6	19	41	1	1	1	2	0	0	0
43	51.2	51.3	9.3	4	4	4	0	0	0	2	0	4	0
44	47.5	62.5	8.0	5	6	8	0	0	1	0	0	0	0
45	66.7	67.5	6.3	4	4	6	1	0	1	2	0	3	0
46	58.8	71.3	90.0	5	7	8	0	0	0	2	0	4	0
47	18.2	13.5	18.0	4	5	7	0	0	0	2	0	2	0
48	40.0	10.8	3.0	4	6	9	0	0	0	2	0	4	0
49	66.3	70.0	77.5	4	4	8	0	0	1	2	0	4	0
50	42.5	92.5	95.0	4	4	5	0	0	0	2	0	4	0
51	5.2	0.0	0.0	12	41	41	1	1	1	0	0	4	0
52	0.0	0.0	0.0	10	34	41	1	1	1	2	0	1	0
53	70.0	43.8	48.8	7	23	41	1	1	1	0	0	4	0
54	0.0	3.8	0.0	4	6	7	0	0	0	2	1	4	0
55	23.5	72.5	62.8	5	6	8	0	0	0	0	0	4	0
56	50.0	51.3	60.5	10	23	41	1	1	1	2	0	3	0
57	46.2	82.5	43.8	4	4	6	0	0	0	2	0	4	3
58	73.7	86.3	36.3	4	4	5	0	0	1	2	1	4	0
59	53.8	76.3	73.8	4	5	7	0	0	0	2	0	4	0
60	87.5	83.8	68.8	4	13	41	0	0	1	0	0	4	0
61	2.0	0.0	3.0	5	7	9	0	0	1	2	0	0	0
62	4.0	4.5	0.0	4	5	6	0	0	1	2	1	4	0
63	11.3	12.0	52.3	11	41	41	1	1	1	2	0	4	0
64	48.8	73.8	72.5	7	41	41	1	1	1	2	0	4	0
65	41.7	66.3	48.8	1	2	5	0	0	1	2	0	0	0