Marieke J.W. Jeuken

The genetics of non-host resistance to the lettuce pathogen *Bremia lactucae* in *Lactuca saligna*

Promotor:	Prof. Dr. Ir. P. Stam Hoogleraar in de plantenveredeling, in het bijzonder de selectiemethoden en duurzame resistentie
Co-promotor:	Dr. P. Lindhout Universitair hoofddocent Leerstoelgroep plantenveredeling
Promotiecommissie:	Prof. Dr. Ir. A. H. C. van Bruggen, Wageningen Universiteit Prof. Dr. Ir. M. Koornneef, Wageningen Universiteit Dr. Ir. F. P. M. Govers, Wageningen Universiteit Dr. Ir. K. Reinink, RijkZwaan BV, De Lier

Marieke J.W. Jeuken

The genetics of non-host resistance to the lettuce pathogen *Bremia lactucae* in *Lactuca saligna*

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. Dr. Ir. L. Speelman
in het openbaar te verdedigen
op woensdag 22 mei 2002
om half twee 's middags in de Aula.

ISBN 90-5808-619-4

Keywords: plant breeding, lettuce, *Lactuca sativa*, *Lactuca saligna*, *Bremia lactucae*, downy mildew, genetics, non-host resistance, QTL, quantitative trait locus, molecular marker, marker assisted selection

STELLINGEN

1. De oude definities van "*Dm*" en "*R*"-genen zijn achterhaald. Het verdient voorkeur om dit onderscheid te baseren op gekloneerde genen.

2. De extra inspanning die nodig is om AFLP merkers co-dominant te kunnen scoren wordt ruimschoots gecompenseerd door het grote gemak waarmee genetische kaarten met behulp van co-dominante merkers geconstrueerd worden.

3. De niet-waard resistentie van *Lactuca saligna* tegen *Bremia lactucae* is de meest waardevolle resistentie voor de sla-veredeling.

4. De Backcross Inbred Line karteringsstrategie kan genen onthullen die verborgen blijven in een F_2 karteringsbenadering (dit proefschrift).

5. De F_2 karteringsstrategie kan genen onthullen die verborgen blijven in een Backcross Inbred Line karteringsbenadering (dit proefschrift).

6. Wetenschappers in de plantenbiotechnologie, die de wereldvoedselvoorziening aangrijpen als verantwoording voor hun onderzoek, kunnen beter condooms uitdelen.

7. Zwanger zijn is een van de meest veelvoorkomende blessures bij vrouwenvoetbal.

Stellingen behorende bij het proefschrift "The genetics of non-host resistance to the lettuce pathogen *Bremia lactucae* in *Lactuca saligna*", door Marieke J.W. Jeuken, in het openbaar te verdedigen op 22 mei 2002, te Wageningen.

Jonge sla

Alles kan ik verdragen,
het verdorren van bonen,
stervende bloemen, het hoekje
aardappelen kan ik met droge ogen
zien rooien, daar ben ik werkelijk hard in.

Maar jonge sla in september,
net geplant, slap nog,
in vochtige bedjes, nee.

Rutger Kopland

Uit de bundel "Geluk is gevaarlijk" van Rutger Kopland, Uitgeverij Van Oorschot.

CONTENTS

1

GENERAL INTRODUCTION

Many crops harbor resistances against potential pathogens. Often resistances are not durable since the pathogen adapts rapidly to its host. As a consequence new potentially durable resistances are searched for in other sources. A good example of a valuable alternative source may be a non-host species that is crossable with the cultivated crop. However, this is a rare possibility that may be hard to identify. Still, such pathosystem exists in the *Lactuca* genus: *Lactuca saligna* is non-host to lettuce downy mildew (*Bremia Lactucae*) and is crossable with cultivated lettuce (*Lactuca sativa*; Bonnier et al. 1992). Lettuce breeders have to put a large effort in breeding for lettuce downy mildew resistance since downy mildew resistances are constantly rendered ineffective by rapid adaptation of the pathogen. Therefore, an alternative resistance from a non-host would be very welcome. The intriguing non-host status of *L. saligna* holds promises to find unknown downy mildew resistance(s) based on possibly new resistance mechanisms. We studied this non-host resistance through a genetical dissection. In this chapter non-host resistance, the crop, the pathogen, the pathosystem, the novel source of resistance, the scope of this thesis and the research plan will be introduced.

PLANT DEFENSE AND NON-HOST RESISTANCE

Plants are continuously exposed to a wide variety of pathogens. However, all plant species are hosts for only a minority of the potential plant pathogens. When the requirements for pathogen growth are met and the pathogen circumvents or tolerates the general defense reactions of a plant, the plant species is a host to the pathogen that may establish a compatible interaction with the plant. Still, in such host-pathogen interactions, the invading pathogen may be recognized by the host and a defense reaction may restrict the infection. Several defense reactions in host-pathogen interactions are known. Surely the best-studied defense reaction is race-specific resistance associated with the hypersensitive response (Flor 1942). Another resistance reaction that is observed for rusts and mildews is partial resistance that reduces epidemical development of the pathogen (Parlevliet 1975, Rubiales and Niks 1995, Singh et al. 1988). Resistance in plants can also be induced locally and systemically through various biotic stresses. Two signal pathways have been characterized for this

induced disease resistance in plants: systemic acquired resistance (SAR) and induced systemic resistance (ISR; Oostendorp et al. 2001, Van Wees et al. 2000).

Besides knowledge of crucial resistance genes involved in the process of pathogen recognition by the plant, many downstream genes in several signal pathways from pathogen recognition to the actual defense reaction are known. Most downstream genes have been characterized in the model plant *Arabidopsis* by mutant analyses, gene isolation and ordering of genes within branches of signal transduction networks (Glazebrook 2001).

All plant species are non-hosts for the majority of the potential plant pathogens. Either the plant does not fulfil the growth requirements of the potential pathogen or the plant perceives the invading pathogen and a general defense reaction follows that is effective in prevention of growth and reproduction of the pathogen (Heath 1981). A plant species is a non-host to a potential pathogen species, when all genotypes of that plant species are fully resistant to all genotypes of that pathogen species (Heath 1981, Niks 1987). Non-host resistance is durable and gives complete resistance, which makes it a very interesting source for applications in resistance breeding. Compared to defense reactions in host-pathogen interactions, not much research has been done on the genetic basis and mechanism of non-host resistance of plants or on the lack of pathogenicity of the non-pathogens (Heath 2001). A few studies on non-host resistance will be briefly illustrated. The INF1 protein of the pathogen *Phytophthora infestans* of potato elicits cell death in the non-host *Nicotiana benthamiana* (Kamoun et al 1998). This resistance is lost if *Phytophthora* does not produce this "non-host avirulence gene". However, the absence of these "non-host avirulence genes" in *Phytophthora* was not sufficient to allow this pathogen to extend its host range to additional *Nicotinana* species (Kamoun et al 1998).

Similarly, *Xanthomonas campestris* pv. *vesicatoria*, pathogen on pepper and tomato, induces a hypersensitive resistance response on non-hosts like bean, soybean, cowpea, alfalfa and cotton. A "non-host avirulence gene" was cloned and appeared to induce resistance in five non-host plant species (Whalen et al 1988). This was demonstrated by the fact that the "non-host avirulence gene", after transfer to several *X. campestris* pathovars, inhibited development of disease symptoms by these transformed *X. campestris* pathovars on their normally susceptible hosts. The hypersensitivity response resistance in bean induced by the non-host avirulence gene segregated as a single incompletely dominant gene.

Another example of non-hosts with resistance genes against "non-host avirulence genes" of inappropriate pathogens is wheat and wheatgrass with two *formae speciales* of *Erysiphe graminis*. Wheat is host for *E. g.* f. sp. *tritici* and non-host for *E. g.* f. sp. *agropyri* and wheatgrass is host for *E. g.* f. sp. *agropyri* and non-host for *E. g.* f. sp. *tritici*. It has been shown that wheat and wheatgrass both have monogenic resistances conferring the hypersensitive response against the inappropriate *E. graminis* formae speciales (Tosa 1992).

These three studies demonstrate that major resistance genes in interaction with non-host avirulence genes may explain the resistance of non-host plant species to

inappropriate pathogens, which is similar to the race-specific resistance in many plant-pathogen interactions. If non-host resistance were completely based on this principle then this would imply that for each inappropriate pathogen at least one resistance gene is present. Consequently, each plant should have thousands of resistance genes, effective against each potential, but inappropriate pathogen species. This seems not very likely. The *Arabidopsis* genome was estimated to contain only about 200 loci that carried nucleotide binding site (NBS) motifs and were analogues of known resistance genes (Meyers et al 1999). Similarly, the *Arabidopsis* genome-sequencing project predicted 174 resistance genes encoding receptor-like kinases with leucine rich repeats (LRR; the Arabidopsis Genome Initiative 2000). For most of these sequences (resistance gene analogues) expression has not even been proven yet, let alone their possible function and specificity. We assume that major resistance genes can only explain part of the non-host resistance to inappropriate pathogens. So what other resistance mechanisms may be the cause of non-host resistances?

Another explanation for non-host resistance is a general defense mechanism not triggered by specific stimuli of the pathogen but by plant metabolites that are toxic to inappropriate pathogens. For example, secondary metabolites, saponines, are implicated to cause the non-host resistance of diploid oat to the inappropriate fungus *Gaemannomyces graminis* var. *tritici,* a pathogen of wheat (Papadopoulou et al 1999).

A third possible explanation of non-host resistance is a non-specific defense reaction induced by non-specific stimuli (Heath 2001). In this model the inappropriate pathogen is not able to suppress a general defense system, causing an incompatible reaction. For example, treatment of non-host plants with a heat shock or with protein synthesis inhibitors before the inoculation of several rust fungi enhanced hyphal growth and haustorium development (Heath 1979). In another study, barley and melon were firstly inoculated with an appropriate powdery mildew that led to penetration and initial growth. When the superficial growth of these pathogens was removed and a second inoculation with an inappropriate powdery mildew was performed, this inappropriate pathogen was now able to grow on the non-hosts (Ouchi et al. 1974).

In a histological study of the inappropriate wheat powdery mildew on barley, it was indicated that there were differences among five barley cultivars in cellular defense reactions from mainly papillae formation till formation of small quantities of colonies (Tosa and Shishiyama 1984). Another study on the prehaustorial resistance of barley to two inappropriate rusts indicated that this resistance was quantitative and was polygenically inherited (Hoogkamp et al 1998, Zhang et al 1994). This is one of the few studies about the genetics behind a general defense system.

In conclusion, we state that more genetical analyses on non-host resistances are needed to understand which resistance mechanisms and genes result in a plant's non-host status. This will also shed more light on the potential durability of these genes, when used in plant breeding.

LETTUCE

Lettuce (*Lactuca sativa*) belongs to the genus *Lactuca* L. (Compositae) that comprises about 100 species. It is classified in the section *Lactuca* L., subsection *Lactuca.*
The lettuce crop has a long history as a leaf and stalk vegetable. The center of origin of cultivated lettuce probably lies in Southwest Asia, in the area around the Euphrates and the Tigris rivers (de Vries 1997, Boukema et al 1990). From there it spread to Egypt where images of lettuce appeared on wall paintings circa 2500 BC (Lindqvist 1960). The Egyptians and later also the Greek and Romans used lettuce not only as a vegetable but also as a sacrificial crop, an aphrodisiac, a soporific and for its good qualities in relation with the digestion (Harlan 1986, Oost 1980). In the Middle Ages lettuce has been introduced in Northwest Europe. Nowadays lettuce is mainly a popular leaf vegetable. It can be consumed all year round because it is cultivated both outdoors and in the greenhouse.
L. sativa has been domesticated from the direct ancestor *L. serriola,* with probably involvement of one or two more wild lettuce species, *L. virosa* and *L. saligna* (Hill et al 1996, de Vries 1997, Koopman et al 2001). These four autogamous lettuce species are compatible for making successful crosses (Thompson et al 1941). *L. sativa* and *L. serriola* are very easily reciprocally crossed. Crosses between *L. sativa* or *L. serriola* as one parent and *L. saligna* or *L. virosa* as the other parent are often accompanied with low seed set, inviable seeds, stunted plants and sterile hybrids (Lindqvist 1960)
The haploid genome of *L. sativa* contains nine chromosomes (2n=18) and about 2.6×10^9 bp (Arumuganathan and Earle 1991). This is 2.6 and 18 times larger than the tomato and *Arabidopsis* genome, respectively. Such a relatively large genome is common for Compositae species (Kesseli and Michelmore 1996).
Since the first gene was identified in lettuce (Durst 1929) many morphological, isozyme and disease resistance loci have been identified (Robinson et al. 1983, Kesseli and Michelmore 1986, Farrara et al. 1987). In the nineties the first linkage map of lettuce based on RFLP and RAPD markers was described from a cross between two cultivars (Kesseli et al. 1994). This map is incomplete as it contains more than 13 linkage groups and major gaps of up to 28 cM. However, several resistance genes have been mapped using this RFLP map as a reference (Witsenboer et al. 1995, Maisonneuve et al. 1994).

LETTUCE DOWNY MILDEW

Bremia Lactucae belongs to the Oomycetes, order Peronosporales and family of *Peronosporaceae.* Oomycetes are organisms that exhibit a filamentous growth habit and are therefore often erroneously referred to as fungi. However, oomycetes are structurally, biochemically and genetically different from all fungal taxa (Campbell 1993). For instance, oomycetes have cell walls predominantly consisting of cellulose, while the major compound in cell walls of true fungi is chitin (Campbell 1993).

Oomycetes belong to the Kingdom of Protista and their closest relatives are heterokont algae and goldenbrown algae (ciliates and dinoflagellates; Campbell 1993, Van de Peer and De Wachter 1997).

The downy mildews (= family of *Peronosporaceae*) are primarily foliage blights that attack and spread rapidly in young, tender green leaf, twig, and fruit tissues. The downy mildews can cause severe losses of 40 to 90% of young plants in short periods of time (Agrios 1997, Lebeda and Schwinn 1994). The most spectacular and catastrophic epidemic was probably the one caused by the downy mildew of grapes, which soon after it's introduction from the USA in 1875, almost completely destroyed the grape and wine industry in Europe and resulted in the discovery of the first fungicide, Bordeaux mixture, in 1885 (Agrios 1997). Although several downy mildews have been studied, from only a few downy mildew species pathogenic variation (=occurrence of races) is known: *Bremia Lactucae* (Crute and Johnson 1976), *Peronospora parasitica* (Holub et al 1994), *Peronospora viciae* (Stegmark 1990, Stegmark 1995, Taylor et al 1989) and *Plasmopara halstedii* (Gulya et al 1991, Mouzeyar et al 1994). *Peronospora parasitica* has been well studied on its model host *Arabidopsis*. Many race-specific resistance genes (*RPP*) are described as well as the *HRT/RPP8* family of resistance genes that confer resistance to both viral and oomycete pathogens (Botella et al 1998, Cooley et al. 2000, McDowell et al. 2000)

Still, one of the best-studied downy mildews on crops is *Bremia Lactucae* on lettuce. *Bremia* is an obligate biotrophic pathogen that grows in and sporulates on the leaves of lettuce and several other *Lactuca* species (Lebeda and Syrovatko 1988). A diagram of the infection of a susceptible lettuce epidermal cell by *Bremia Lactucae* is presented in Figure 1.

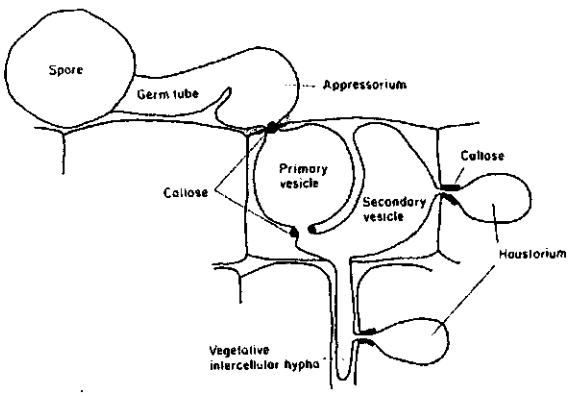


FIGURE 1. A representation of the infection of a susceptible lettuce epidermal cell by *Bremia lactucae* (after Ingram et al 1973).

Bremia is dispersed primarily by windblown spores. It reproduces mainly asexually and occasionally sexually. In *Bremia* sexual reproduction occurs when isolates of opposite sexual compatibility types (B1 and B2) are in close proximity and mate, yielding large numbers of oospores (Crute 1992). Many races of *Bremia* are known from lettuce cultivars as well as from wild lettuce species like *L. serriola* (Crute and Johnson 1976, Lebeda and Boukema 1991). For instance, in Europe at least 24 commonly found races on *L. sativa* have been described from 1964 to 1999 (Van Ettekoven and Van der Arend 1999, Lebeda and Schwinn 1994).

PATHOSYSTEM AND MONOGENIC RACE-SPECIFIC RESISTANCE

Bremia Lactucae is pathogenic on cultivated lettuce and on some wild lettuce species (*L. serriola, L. virosa, L. altaica* and *L. degreana*; Lebeda and Syrovatko 1988, Lebeda and Boukema 1991). It is an important disease in lettuce worldwide and resistance to *Bremia* is one of the most important breeding goals in all lettuce types (Reinink 1999). The lettuce species *L. sativa, L. serriola* and *L. virosa* show a large variation of resistances to downy mildew (Bonnier et al 1992). The most common and exploited resistance is qualitative and is under genetic control of single dominant genes, *Dm* genes (Downy mildew) or *R*-genes. It is generally agreed among phytopathologists, geneticists and lettuce breeders that when it is shown that this resistance is explained by one single gene, it is designated "*Dm* gene". Until that time, the resistance is named "*R*-gene". Plants harboring *Dm* genes are resistant to some but susceptible to other *Bremia* races because of a classical gene-for-gene interaction (Flor 1942). The combination of a *Dm* gene and a corresponding avirulence gene of *Bremia* results in an incompatible interaction, which is associated with a hypersensitive response of the host (Crute & Johnson 1976). Nineteen *Dm* genes have been identified from cultivated germplasm sources or closely related species like *L. serriola* (Landry et al.1987, Crute 1992, Bonnier et al. 1994, van Ettekoven and van der Arend 1999). Like dominant monogenic resistances in other plant species, the *Dm* genes are distributed in clusters over the genome (Kesseli and Michelmore 1996). Three major clusters of resistance genes have been located on Chromosome 1, 2 and 4 (Kesseli et al.1994). Remarkably, the resistance gene cluster at the *Dm3* region, which has a size of at least 3.5 Mb, is very large compared to other resistance clusters in other species (Meyers et al. 1998a). The *Dm3* gene has been cloned from a highly divergent family of 22 resistance gene homologues. The *Dm3* gene belongs to a class of resistance genes with a nucleotide binding site (NBS) domain and leucine-rich repeats (LRR) (Meyers et al 1998a, Michelmore and Meyers 1998). Sequence comparison of *Dm3* and eight resistance gene homologues indicated that diversifying selection has resulted in increased codon variation in the LLR region (Meyers et al. 1998b). These results support a "birth and death"model that supposes that the variation in the LRR regions, is due to mutations rather than to events like intergenic unequal crossing over and gene conversions (Michelmore and Meyers 1998).

During half a century of commercial resistance breeding of lettuce, the emphasis has invariably been on dominant *Dm* genes (Crute 1992, Reinink 1999). Over nineteen *Dm* genes have been introgressed into commercial cultivars. Unfortunately, *Dm* genes have been rendered ineffective by rapid adaptation of the pathogen (Lebeda and Zinkernagel 1999). For example, in 1987 a new race of *Bremia*, NL16 spread over large parts of Europe in a relatively short period of time and remained the dominant race for about ten years. A new resistance gene effective against race NL16 was introduced: *R18* (Reinink 1999). However, after 1995 many new races of *Bremia* have been found in Europe on cultivars containing *R18* and later also on cultivars with newly introduced resistances *R36*, *R37* and *R38*. This illustrates that the effectivity of *Dm* genes does not last very long.

L. SALIGNA, A LETTUCE DOWNY MILDEW NON-HOST

Among the non-hosts of lettuce downy mildew, *Lactuca saligna* is the best-studied *Lactuca* species. *L. saligna* can be crossed with cultivated lettuce and is completely resistant to *Bremia* (Norwood 1981, Gustafsson 1989, Lebeda and Boukema 1991, Bonnier et al.1992). Therefore, it can be considered a non-host. In histological studies it was observed that in *L. saligna*, a smaller proportion of the primary vesicles of *Bremia* (race NL16) formed a secondary vesicle as compared to a *L. sativa* cultivar with a *Dm* gene effective against this race (Sedlarova et al 1999). Furthermore, *L. saligna* accessions varied in resistance reactions like necrosis formation after *Bremia* inoculation (Lebeda and Reinink 1994). It may be that this hypersensitivity is due to a resistance acting in a gene-for-gene like manner, like *Dm* genes and avirulence genes. Some *L. saligna* accessions, however, do not show necrosis. The latter resistance may be ascribed to an alternative resistance mechanism.

LETTUCE BREEDING FOR RESISTANCE TO *BREMIA LACTUCAE*

As described above, lettuce breeders have focused mainly on *Dm* genes for resistance to *Bremia*, although these are not effective for a long time. Breeding for other resistance like quantitative resistance has been attempted (Crute and Norwood 1981, Eenink 1981, Gustafsson 1989, Lebeda 1990, Reinink 1999). However, no successful cultivars have been released with increased level of this resistance (Reinink 1999). Quantitative resistance is a difficult trait to select for and therefore difficult to introgress into cultivars, due to it's smaller effects and often polygenic inheritance. If in some cases quantitative resistance was introgressed, the obtained resistance levels were too low to make the cultivar successful.

A good alternative strategy for breeding for resistance to *Bremia* could be the introduction of novel types of resistance from the wild species *L. saligna*, a non-host for *Bremia*. This source of resistance has occasionally been exploited for *Dm* genes

that have been rendered as ineffective as the *Dm* genes in *L. sativa* sources (Lebeda and Zinkernagel 1999). But *L. saligna* has not been exploited and introgressed earlier yet for the novel types of resistance that may explain the non-host status of *L. saligna*. Two reasons account for this. Firstly, interspecific crossing barriers result in low seed set, low viability of those seeds, stunted plants and sterile hybrids. Secondly, the resistance is very difficult to select for with classical breeding tools, which indicates a quantitative character and/or a polygenic inheritance of this resistance.
However, new possibilities for research are offered with the recent developments in Marker Assisted Selection, QTL mapping and high throughput molecular marker techniques. By using these techniques plants can be screened and selected on basis of genotype instead of phenotype. The intensive and crucial phenotyping tests can be postponed till the most promising genotype has been obtained or till the introgression program has reached the optimal stage for phenotyping.

SCOPE OF THIS THESIS

This thesis presents a study on the genetical dissection of the resistance of *L. saligna* based on two accessions, in order to locate the resistance gene(s) that confer complete resistance to all *Bremia* races on the *Lactuca* genome. This is of scientific interest as not much is known about the character and inheritance of the resistance(s) that renders a species a non-host. It is also of breeders' interest to find out which genes are necessary to introgress from the non-host to the host in order to transform it into a non-host.
In order to map the genes responsible for the resistance of *L. saligna* to *Bremia*, we selected breeding material of an F_2 population and a BC_1 population from two crosses of two *L. saligna* accessions with a susceptible *L. sativa*. Most emphasis was put on the progeny of the *L. saligna* accession with the largest, most viable and fertile F_2 and BC_1 populations (Figure 2). All the progeny of this most successful cross is described in Chapters 2, 3, 4 and 5. The progeny of the cross with a different *L. saligna* accession with a smaller F_2 population is described in Chapters 2 and 6.
We planned two strategies to unravel the genetics of the resistance of *L. saligna*. The effectiveness of the two strategies will be compared. For both strategies a linkage map based on molecular markers is required. To develop a molecular marker map of a *L. saligna* × *L. sativa* cross, we performed AFLP analyses on both F_2 populations and used both data sets to assemble an integrated map (Chapter 2).

FIGURE 2. *Lactuca sativa* cv "Olof" and *Lactuca saligna* CGN 5271

The first strategy is a classical F_2 mapping population strategy, in which all F_2 plants are genotyped with molecular markers and phenotyped by *Bremia* disease tests (Figure 3). In a QTL mapping procedure genotypic and phenotypic data are combined, to identify genomic regions with genes responsible for the resistance (Chapter 3 and 6).

The second strategy is the mapping of resistance genes in a set of Backcross Inbred Lines (BIL); each BIL harbors a single *L. saligna* introgression fragment in a *L. sativa* background, while all BILs together cover the total *L. saligna* genome. The development of a set of BILs by repeated backcrossing and Marker Assisted Selection is described in Chapter 4. The first results of *Bremia* disease tests on a selection of BILs are described in Chapter 5. Comparison of the two strategies and the detection of resistance genes are discussed in the general discussion (Chapter 7).

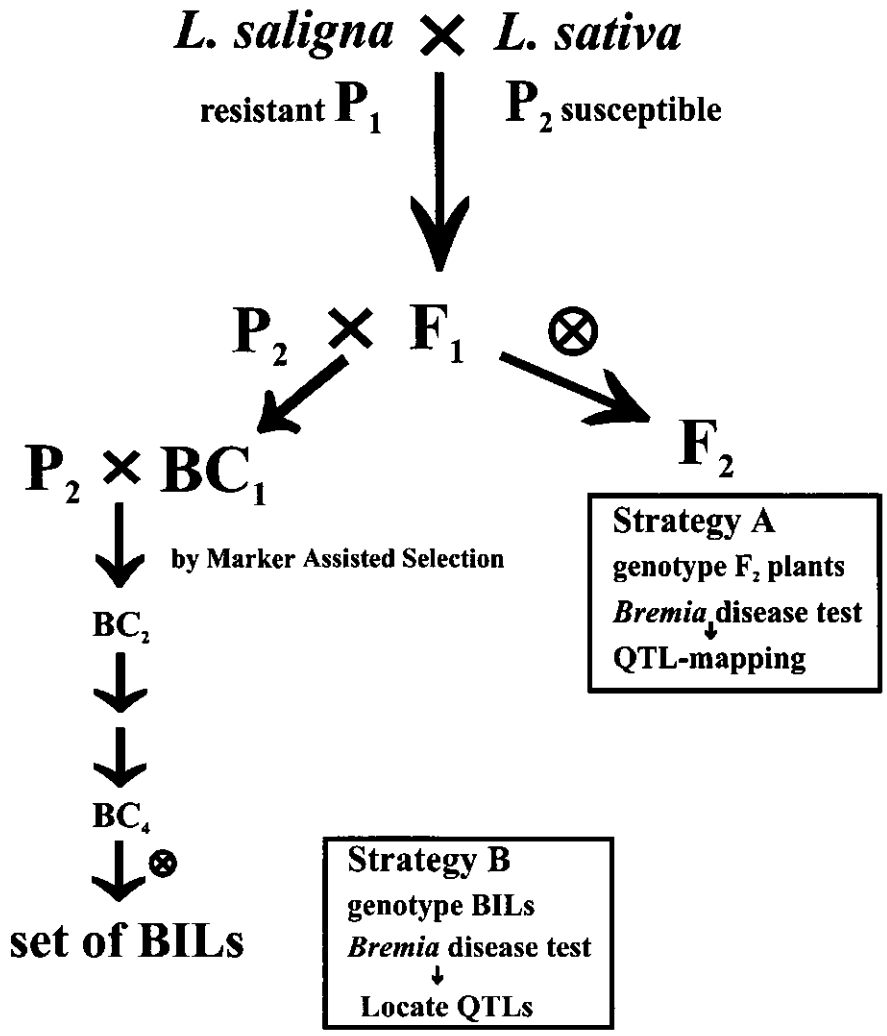


FIGURE 3. Working plan for the genetical dissection of the resistance of *L. saligna* to *Bremia lactucae* by two strategies. ⊗ = selfing

2

AN INTEGRATED INTERSPECIFIC AFLP® MAP OF LETTUCE (*LACTUCA*) BASED ON TWO *L. SALIGNA* × *L. SATIVA* F_2 POPULATIONS

Marieke Jeuken, Rik van Wijk, Johan Peleman and Pim Lindhout

Published in Theoretical and Applied Genetics 103 (2001): 638-647

AFLP markers were obtained with twelve *Eco*RI/*Mse*I primer combinations on two independent F_2 populations of *L. sativa* × *L. saligna*. The polymorphism rates of the AFLP products between the two different *L. saligna* lines was 39 %, between the two different *L. sativa* cultivars 13% and between the *L. sativa* and *L. saligna* parents on average 81%. In both F_2 populations segregation distortion was found, but only Chromosome 5 showed skewness that was similar for both populations. Two independent genetic maps of the two F_2 populations were constructed that could be integrated due to high similarity in marker order and map distances of 124 markers common to both populations. The integrated map consisted of 476 AFLP markers and twelve SSRs on nine linkage groups spanning 854 cM. The AFLP markers on the integrated map were randomly distributed with an average spacing between markers of 1.8 cM and a maximal distance of 16 cM. Furthermore, the AFLP markers did not show severe clustering. This AFLP map provides good opportunities for use in QTL mapping and marker-assisted selection.

Our knowledge on the structure and function of plant genomes is rapidly expanding by the fast development of techniques in molecular biology like automated sequencing, DNA library construction and screening, and DNA marker technologies. The new research field about maintaining, ordering and using all this genome information is designated as "Bioinformatics". This covers fundamental research topics like gene organisation and synteny among genomes. A more applied field is plant breeding where bioinformatics will facilitate marker-assisted selection programs with most emphasis on quantitative traits.

The molecular information of a plant genome is usually presented in a framework of a genetic linkage map. To create such a genetic map informative markers need to be developed and screened on a segregating population. To this end, markers of several types are available. Former genetic maps of many plant species are mainly constructed with RFLPs as markers. The advantages of RFLPs are the locus specificity and codominant inheritance. The disadvantage is that the technology is time consuming, laborious and costly. Nowadays, new DNA marker technologies are available, which are PCR based, need less template DNA and are less laborious. Examples of commonly used PCR based marker technologies are CAPS (Konieczyn & Ausubel 1993), SSR (Van de Wiel et al. 1999) and AFLP (Vos et al. 1995). CAPS and SSR are reliable markers with potentially many alleles and hence a codominant inheritance. These markers are mainly used as easy applicable markers for specific loci. Their disadvantage is the *a priori* sequence information that is required to design the locus specific primers. In contrast, the AFLP technique does not require *a priori* sequence information and combines the advantages of RFLP markers with the advantages of PCR. AFLP markers are efficient and reliable and can be used across species like is shown for tomato, potato, barley and maize (Haanstra et al. 1999; Van Eck et al. 1995; Qi et al 1998; Vuylsteke et al.1999).

These new marker technologies allow the efficient construction of high-density maps, which have several applications in genetics and breeding. For instance, comparison of the synteny among genomes of related species or genera as shown for *Solanaceae*, cereals and *Brassica* species (Livingstone et al. 1999, Gale and Devos 1998, Hu et al 1998). This allows the construction of integrated genetic maps among species or within genera and so to make comparisons between related genera (Qi et al. 1996, Sebastian et al. 2000).

Furthermore, genetic maps are essential to locate the genes that are involved in the expression of traits. This can easily be done for simple heritable traits based on one gene, but also for complex traits which are based on more genes (QTLs). In the latter case large segregating populations (n>100) are required to unravel the number of loci involved in the trait.

When the map positions of important genes are known indirect selection of plants, bearing the useful genes, can take place at the DNA level on the basis of flanking markers linked to the genes of interest. This so called "marker-assisted selection", has high potentials in plant breeding (Bernatsky & Tanksley, 1989; Lande & Thompson, 1990; Knapp 1998).

In lettuce, a genetic map is available, which is based on an intraspecific cross "Calmar × Kordaat" and consists of thirteen major and four minor linkage groups spanning a total length of 1950 cM (Kesseli et al. 1994). It consists mainly of RFLP and RAPD markers with an average spacing of 6.1 cM and major gaps up to 28 cM. This map has been used to map *Dm* genes and other disease resistance genes (Okubara et al. 1994; Witsenboer et al. 1995; Maisonneuve et al. 1994).

We are interested in *Lactuca saligna* (wild lettuce) as a source for resistance to downy mildew (*Bremia lactucae*). The resistance from *L. saligna* is probably not race-specific and therefore probably controlled by a different resistance mechanism than

the gene-for-gene resistance mechanism of introgressed race-specific resistance genes (*Dm* genes) in *L. sativa* (Bonnier et al. 1992, Lebeda & Reinink 1994). *L. saligna* and lettuce *(L. sativa)* are crossable but due to their genetic distance the success of crosses is low, which results in reduced germination, vigour and fertility of the progenies (De Vries, 1990; Koopman et al. 1998). To map the downy mildew resistance in *L. saligna* we aimed at constructing a genetic map based on a *L. saligna* × *L. sativa* cross.

In the present study two different independent F_2 populations of *L. saligna* × *L. sativa* crosses were generated of which a dense integrated genetic linkage map was constructed mainly based on AFLP markers.

MATERIALS AND METHODS

Plant material

Two F_2 mapping populations were generated for this study. The parents of Population A were *L. saligna* CGN 5271 as female parent, and *L. sativa* cv "Olof" , a butterhead cultivar as male parent. The parents of Population B were *L. saligna* CGN 11341 as female parent and *L. sativa* cv "Norden", a butterhead cultivar as male parent. The two *L. saligna* parents had a very distinct morphology. There is no information available on their geographical origin.

The F_2 populations consisted of 126 plants for Population A and 54 plants for Population B. Each F_2 population was derived from a randomly chosen single F_1 plant.

Populations A and B were supplied by the breeding companies Nickerson-Zwaan and Rijk Zwaan, respectively.

DNA isolation

Leaf material was collected from eight weeks old F_2 plants that were grown in the greenhouse. Genomic DNA was extracted from frozen leaves according to the procedure as described by Van der Beek et al. (1992) with some minor modifications: after hooking the DNA out of the isopropanol mixture, the DNA was washed overnight in 76 % ethanol and 10 mM NH_4Ac, dried and dissolved in 200 µl sterile TE buffer (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA).

TABLE 1. List of primer combinations used for AFLP analyses

	M48 CAC	M49 CAG	M54 CCT	M58 CGT	M59 CTA	M60 CTC
E35 ACA	×	×			×	×
E38 ACT			×			
E44 ATC	×	×				
E45 ATG	×	×				
E49 CAG				×		
E51 CCA		×				
E54 CCT	×					

The names and the last three selective nucleotides of the primers are shown. For pre-amplification, the same primers were used without the last two selective nucleotides.

AFLP analysis
The AFLP procedure was performed according to the two step amplification as described by Vos et al. (1995) using the enzyme combination *Eco*RI/*Mse*I. A total of twelve primer combinations, selected from a study on informative primer combinations in lettuce (Van Wijk, personal communication) were applied. The following seven primer combinations E44M48, E35M48, E49M58, E54M48, E45M49, E51M49, E38M54 were applied to all F_2 plants of both populations, while five other primer combinations, i.e. E45M48, E35M60, E44M49, E35M49 and E35M59, were only applied to 90 F_2 plants of Population A (Table 1).

AFLP marker nomenclature and analysis of gel images
AFLP markers were designated with the name of the two primers (e.g. E35M48) used to amplify the DNA, followed by the molecular size as number of nucleotides of the amplification product as estimated from the mobility in the gel compared to a size standard. In case two different bands from the same primer combination were almost but not exactly identical in size, their marker names were extended with "a" for the larger fragment and "b" for the smaller one. The other extensions in the marker names referred to the specific parent that showed this amplification product (see legends of Figure 1).
The scoring of the AFLP markers produced with primer combinations E44M48, E35M48, E49M58, E54M48, E45M49, E51M49, E38M54 were mainly based upon the presence or absence of the amplification product (e.g. dominant scoring). Only when intensity differences of amplification products allowed distinguishing between homozygotes and heterozygotes, the markers were scored codominantly. All markers generated with these seven primer combinations were scored twice, and discrepancies were resolved. The AFLP markers in Population A produced with primer combinations E45M48, E35M60, E44M49, E35M49, E35M59 were predominantly scored codominantly using proprietary software (developed at Keygene).

Calculation of polymorphism rates based on AFLP data
All amplification products obtained by using the 12 primer combinations on all four parents were counted. The polymorphism rate was defined as the number of segregating amplification products divided by the total number of amplification products within the size range of 60-590 basepairs.

SSR primers
The following SSR primer pairs obtained from Van de Wiel and developed on *L. sativa* were tested on the four parent lines: LsA001, LsA002a, LsA003, LsA004a, LsA006, LsB101, LsB102, LsB104, LsB105, LsB106, LsB107, LsB108, LsB110, LsB111a, LsB71f6r, LsB8, LsD035, LsD046, LsD101, LsD103a, LsD106G, LsD107G, LsD108, LsD109, LsD110a, LsE003a, LsE006, LsE009, LsE011, LsE018, LsF018, LsG001G and LsH001 (Van de Wiel et al. 1999). Only in case both parents showed unique alleles, the F_2 populations were screened for segregation of such SSR marker.
The following additional SSR primer pairs obtained from Michelmore (Davis, California, USA) were tested and showed polymorphism among four parental lines and in the two F_2 populations: L1722, L1723, L222, L2211, L2278, L2524#2 and L317. More SSRs obtained from Michelmore were tested on the parental lines, but did not show unique alleles for each parent and were not tested on the F_2 populations (results not shown).

SSR analysis
Amplification of SSRs was performed in 20 µl PCR reactions containing 20 ng template DNA, 0.4 U Taq polymerase, 40 ng of both primers, 2 µl 10x reaction buffer (same as used in AFLP analysis) and 0.1 mM of all four dNTPs. The following PCR program was used: 1 min 94°C, 40 cycle of 45 s of annealing temperature, 1 min 45 s of extension at 72°C, 45 s of denaturation 94°C with a final step of 3 min at 72°C. PCR products were run on 3% agarose gels to separate amplification products with larger size differences. Otherwise, they were

separated on denaturing polyacrylamide gels with conditions similar to AFLP analysis to separate amplification products with lengths between 80 and 500 nucleotides.
In case more amplification products were obtained (the SSR was multilocus), an extension to the original name was given with first the specification of the parent and than the estimated fragment size.
The SSRs were scored based upon the presence or absence of the amplification products of the parents. SSRs were scored codominantly in case both parents showed unique alleles.

Linkage analysis and map integration
To analyse the scored markers, segregation distortion tests and linkage analyses were performed by using JoinMap 2.0 (Stam and Van Ooijen 1995) on each mapping population.
For the F_2 segregation ratios a χ^2 test for skewness was performed with a threshold level for significance of 0.5%. For Population A markers codominantly scored were tested against the 1:2:1 ratio, referring to homozygous *L. sativa*: heterozygous: homozygous *L. saligna*. Markers dominantly scored were tested against the 3:1 ratio, representing homozygous *L. sativa* plus heterozygous: homozygous *L. saligna* or homozygous *L. saligna* plus heterozygotes: homozygous *L. sativa*.
For linkage analysis markers were assigned to linkage groups by increasing the LOD score for grouping with steps of one LOD unit. The calculations of the linkage maps were done by using all pairwise recombination estimates smaller than 0.45, LOD scores higher than 0.01 and Kosambi's mapping function.
After the calculation of a map for each population the two maps were integrated by using JoinMap 2.0 after merging the pairwise recombination frequencies and the corresponding LOD scores of both populations. Again, linkage groups were assigned by increasing the LOD score for grouping with steps of one LOD unit. Map distances were calculated using Kosambi's mapping function, pairwise recombination estimates smaller than 0.45 and LOD scores higher than 0.5 to save calculation time.
Markers, that could not reliably be fitted by JoinMap due to conflicting recombination estimates but that had a LOD score for linkage with another marker higher than or equal to 10 or 5 combined with a recombination frequency smaller than or equal to 5 or 10 % were manually placed on the map on the most likely position and given an extension "!".

RESULTS

Plant material
To establish a reliable map it was aimed that the population size was more than 100 F_2 individuals. Population A consisted of 162 seeds, which germinated well and resulted in 126 full-grown F_2 plants. Population B had a much lower germination rate of 42 %, resulting in only 54 F_2 plants out of 130 seeds.
The variation in the morphology of the F_2 plants of both populations was very high. The fertility of the F_2 plants was very low compared to the parent plants. In both populations 37% of the F_2 plants were sterile. The rest of the F_2 plants varied in seed set, ranging from a dozen to more than 100 seeds per plant.

TABLE 2. Specificity and number of AFLP amplification products generated with twelve primer combinations

12 primer combinations	*L. sativa* specific	Olof specific	Norden specific	*L. saligna* specific	*L. saligna* A specific	*L. saligna* B specific	Constant bands	Total # of bands
Average	39	3	3	28	10	8	18	109
Total	473	40	33	338	119	93	221	1317

L. sativa specific means that the amplification product is found in *L. sativa* cv "Olof" and in *L. sativa* cv "Norden", while Olof specific means that the amplification product is found in *L. sativa* cv "Olof" only and not in Norden. Similarly for *L. saligna* specific, *L. saligna* **A** specific and *L. saligna* **B** specific amplification products. Constant bands are amplification products found in all four parents.

AFLP analysis and polymorphism rates

By analysing 12 primer combinations on all four parents 1317 different amplification products were generated. From these AFLP amplification products 1096 were segregating in the F_2 populations and ascribed to one of the parents as they showed to be parent specific (Table 2). The polymorphism rate between *L. sativa* and *L. saligna* in Population A and B was 81.4 % and 80.9 % respectively, the polymorphism rate between *L. sativa* cv "Olof" and *L. sativa* cv "Norden" was 13.4 % and between *L. saligna* A and *L. saligna* B 38.5%. Twenty-nine amplification products were excluded from the analyses, because they could not be ascribed to only one parent.

On average, with each primer combination 109 amplification products were produced of which 45 (=39+3+3) were detected only in *L. sativa* and 46 (=28+10+8) were detected only in *L. saligna* (Table 2).

In Population A, screened with all twelve primer combinations, 482 polymorphisms were scored. Fifty present of the segregating amplification products showed nearly identical mobility on the gel. Therefore they could not be scored reliably and were not included in the analyses. The other fifty percent of the segregating amplification products were scored unambiguously. Population B was analysed with seven primer combinations and yielded 294 scorable polymorphisms.

SSR analysis

From the 76 SSR primer pairs tested, only four of them, i.e. L317, L222, L2211 and LsB104 were scored codominantly. Most of the other SSR primer pairs yielded an amplification product in the *L. sativa* parent only, which resulted in a dominant scoring. The rest did not show any polymorphism between the parents.

Genetic linkage map and segregation distortion of Population A

In Population A 482 AFLP markers and 12 SSR markers were scored and used for map calculation. These markers were assigned to linkage groups at a LOD threshold of 6.0. The genetic map derived from Population A contained 412 markers (83% of the total number of markers) on ten linkage groups covering a total map length of 895 cM (data not shown).

In this F_2 population 25% of the loci showed segregation distortion. Linkage Group 7 showed an average skewed ratio of 37 : 44 : 8 instead of 1 : 2 : 1 over its entire length severely favouring *L. sativa* alleles. Furthermore, skewness of similar severity was

observed at one of the ends of the Linkage Groups 4, 6 and 9, all in favour of *L. sativa* alleles (Table 3). An average segregation distortion of 3 : 43 : 39 favouring *L. saligna* alleles was found distal on Linkage Group 4 and a similar severe skewness was found on Linkage Group 5 (Table 3). Besides skewness also an excess of heterozygotes was found with an average ratio of 20:62: 4 on Linkage Group 8 at 21-45 cM.

Genetic linkage map and segregation distortion of Population B

In the smaller F_2 Population B, 294 AFLP markers and 8 SSRs were used for map calculation. The markers were assigned to linkage groups at a LOD threshold of 4.0 resulting in a map of 13 linkage groups (data not shown). The alignment of the maps of both populations revealed that the common markers fell in the same linkage groups. Based on the alignment six groups in Population B corresponded with three groups of Population A, as Population A contained several bridging markers that were not scored in Population B. Consequently, the six groups in Population B were merged into three groups.

Fixed order files from Population A with common markers at ≥15 cM intervals were used to generate a genetic map of Population B. This resulted in a map of 223 markers (74% of total number of markers) on ten linkage groups covering a total map length of 627 cM.

Two regions on Linkage Group 5 and 6 in population B showed severe skewness favouring both *L. saligna* alleles (Table 3).

Integrated map

The two linkage maps, generated from the two F_2 populations were very similar with respect to marker order and distance for each linkage group. Consequently, an integrated map, comprising markers of both populations, was constructed. The markers were assigned to nine linkage groups at a LOD threshold of 6.0. This corresponds with the chromosomal number of lettuce. The numbers given to the

TABLE 3. Observed segregation distortion, per population and per linkage group

Linkage Group	Region in cM	Favouring alleles of
Population A		
4	0-7	*L. saligna*
4	116-142	*L. sativa*
5	0-41	*L. saligna*
6	73-84	*L. sativa*
7	0-75	*L. sativa*
9	0-31	*L. sativa*
9	78-101	*L. sativa*
Population B		
5	0-37	*L. saligna*
6	0-9	*L. saligna*

linkage groups correspond with the group numbering used for the "Calmar × Kordaat" map (Kesseli et al 1994) with exception of Group 6 in this map that corresponds with Group 12 in the "Calmar × Kordaat" map. We follow the nomenclature for chromosomal numbers as proposed by Michelmore and Van Wijk for the "Calmar × Kordaat" map, which allows the alignment of both maps with other maps of lettuce with markers in common (Michelmore & Van Wijk in preparation).
Over the two populations 533 different markers were scored, of which 488 (=92%) were mapped covering a total map length of 854 cM (Fig.1). From these mapped markers, 124 (25%) were scored in both populations and were located on similar map positions. Therefore, they were considered as common markers. Out of 488 mapped markers twelve were SSR markers of which four were scored codominantly.
The distribution of the markers over the map was random and no clear clustering of markers was observed except for a small cluster in the centre of Chromosome 6 where 17 markers were present at an interval of 0.6 cM.
The average spacing between markers (including markers at the same position) was 1.8 cM and the largest gap between two markers was 16 cM.

Co-linearity between the three maps

Both individual maps had ten linkage groups, while the integrated map had nine linkage groups corresponding to the nine chromosomes of lettuce (Table 4). The two linkage groups representing Chromosome 8 in both individual maps were not joined because the linkage between the distal markers E49M58-258sal, E38M54-140sal and E51M49-245sal was lower than the LOD threshold for grouping (LOD 6.0 and 4.0 in Population A and B, respectively). In the integrated map the two groups were joined because the linkage between the distal markers of the two groups was above the LOD threshold for grouping (LOD 6.0). This was due to the summed number of genotypes from both populations, which increases the LOD score for linkage between these markers (Fig. 2). The other eight linkage groups were similar in marker order and distance among the maps. The only exception is marker E54M48-216, which was mapped in Population A on Chromosome 6 and in Population B on Chromosome 4. Apparently, this is not a common marker. On the integrated map their parent specific extensions "satA" and "satB" distinguish these markers.
Furthermore, through integration of the maps the number of population specific markers dropped from 385 to 363. These lost specific markers were "Population B"-specific markers that had a LOD score higher than 4.0 but lower than 6.0 and therefore could not meet the criteria for the integrated map.
The marker order between all three maps was highly similar with some minor rearrangements of marker orders at small map intervals of less than five cM (For example, in Chromosome 8 in Fig.2). As the accuracy of the location of the markers in the maps is about five cM, these smaller differences are probably due to errors in the data set.
The genetic distances between the maps were similar although the length of the map of Population B is 30 % smaller than the length of the map of Population A. By counting the map distances from the most distal common markers to the end of the

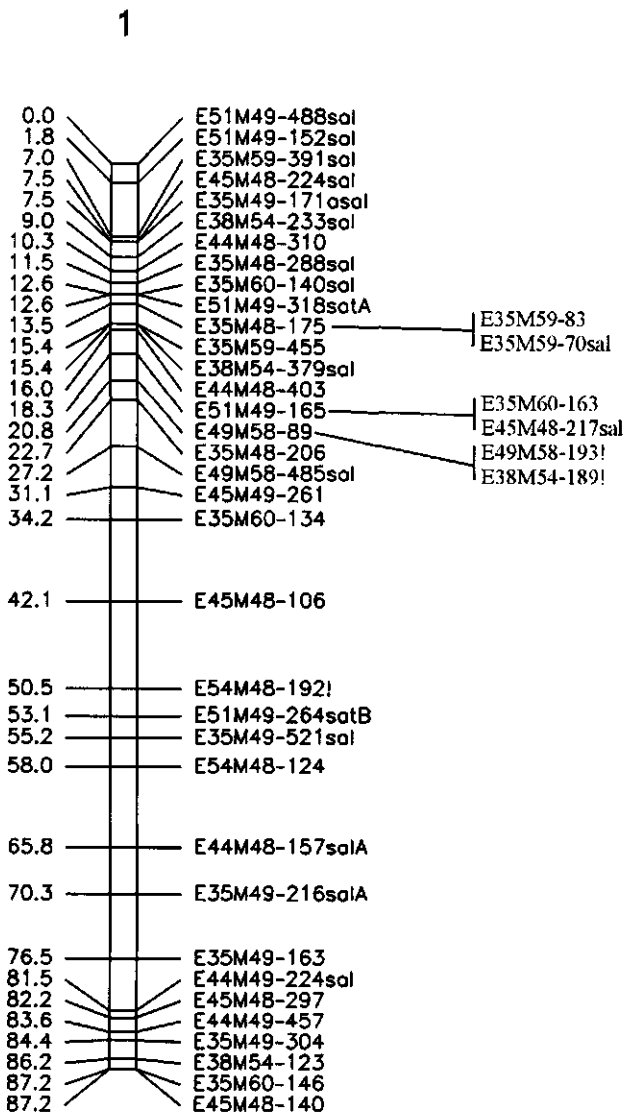


FIGURE 1. An integrated map based on two interspecific F_2 populations between *L. saligna* and *L. sativa*. Chromosome 4 is split up because of its length. Markers with no extension only give an amplification product in *L. sativa*. The extensions satA, satB, sal, salA, and salB represent markers that only give amplification products in respectively *L. sativa* Olof, *L. sativa* Norden, *L. saligna*, *L. saligna* A and *L. saligna* B. The extension ! means that a marker is placed there manually at the most likely position with restrictions to recombination frequency and the LOD score (see results). When three or more markers mapped on the same position they were put aside.

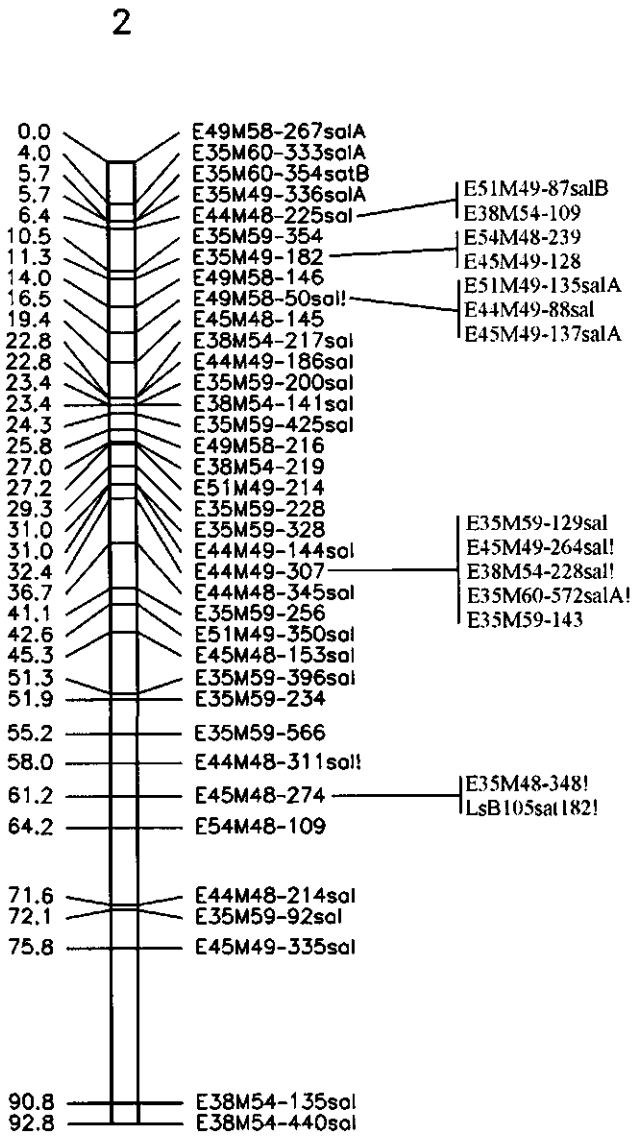


FIGURE 1. Continued

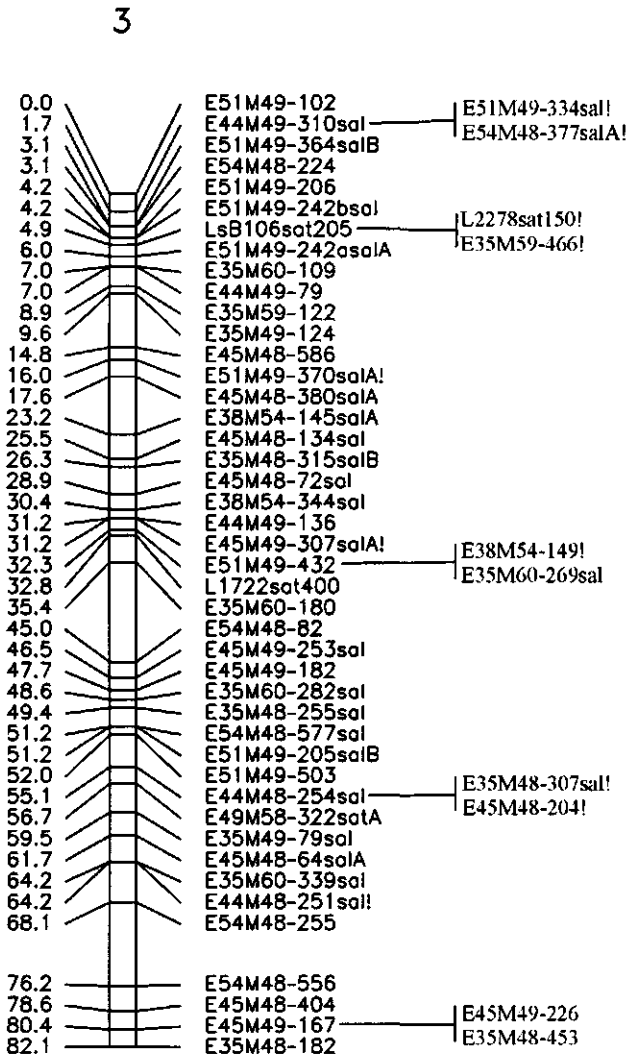


FIGURE 1. Continued

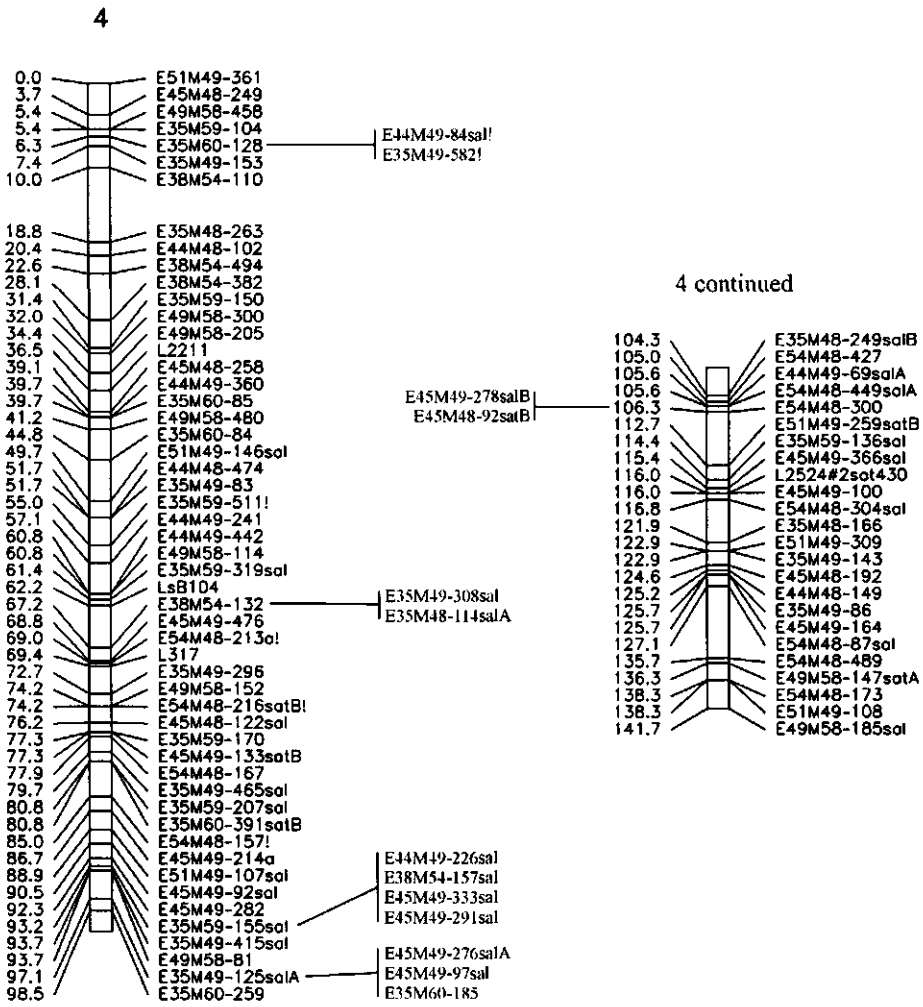


FIGURE 1. Continued

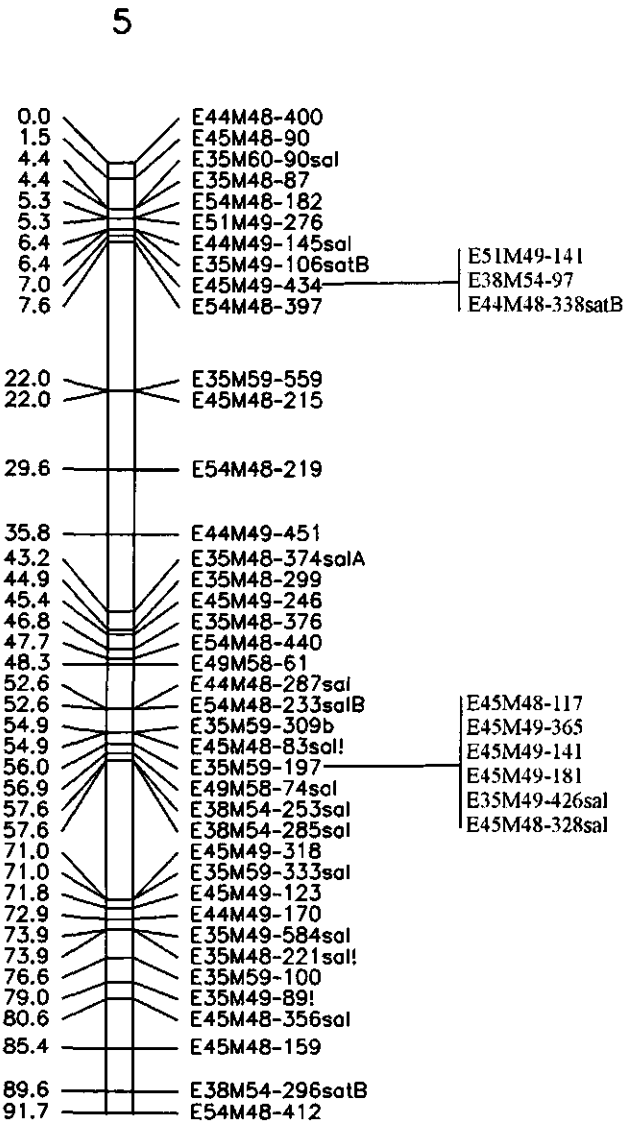


FIGURE 1. Continued

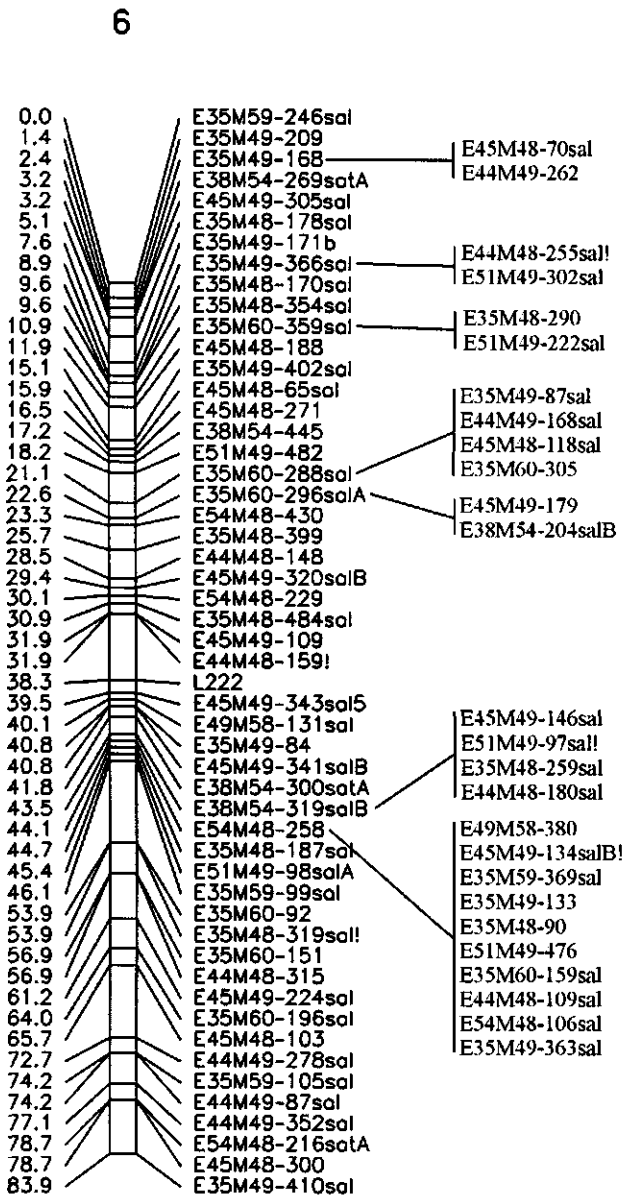


FIGURE 1. Continued

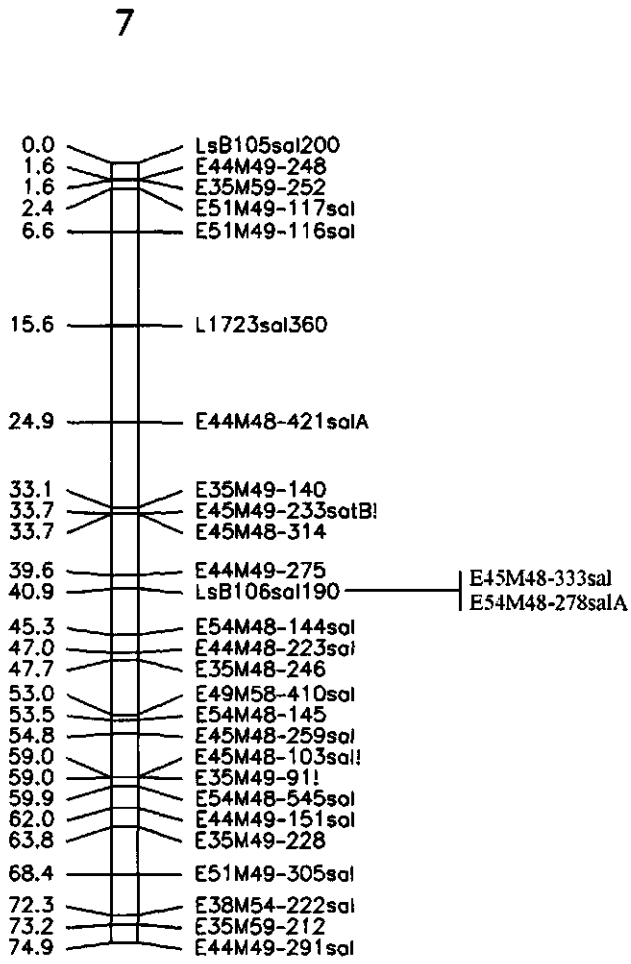


FIGURE 1. Continued

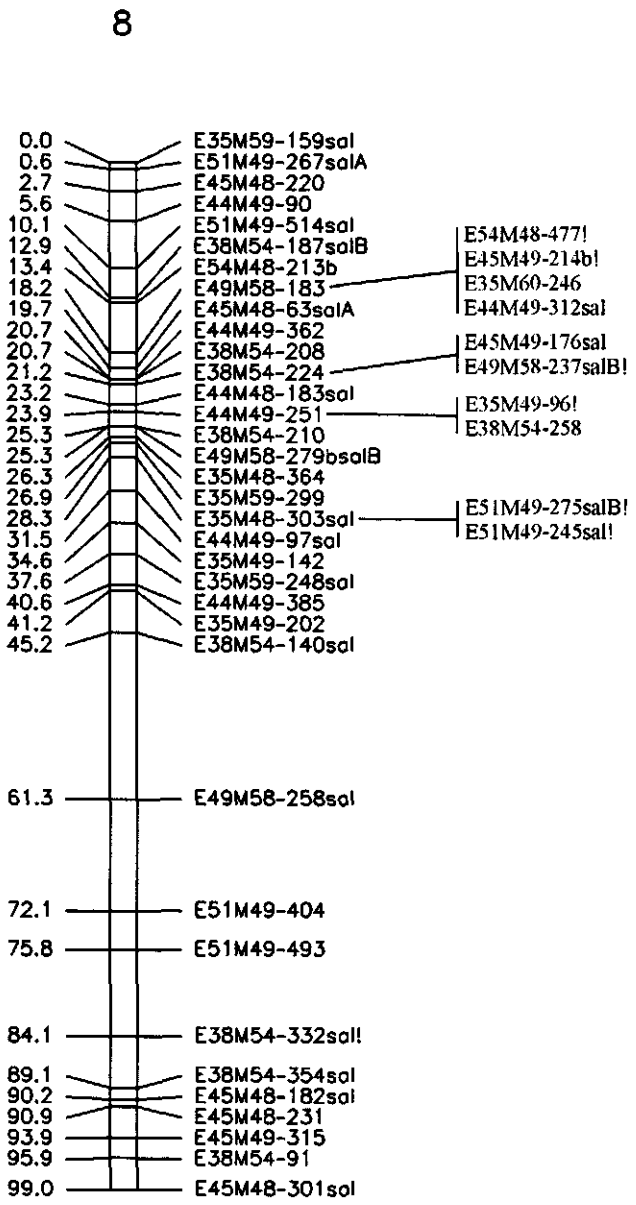


FIGURE 1. Continued

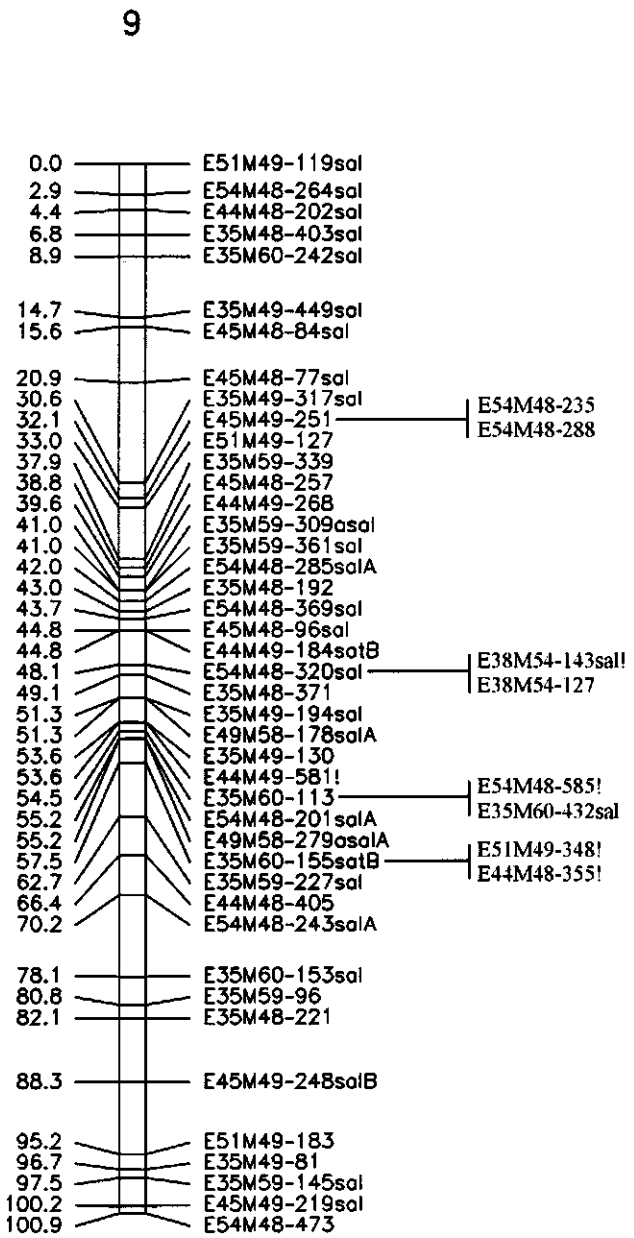


FIGURE 1. Continued

TABLE 4. Comparison of maps of Population A, B and the integrated map

	Map of Population A	Map of Population B	Integrated map
# of linkage groups	10	10	9
Total map length (cM)	895	627	854
# of common markers[1]	124	124	124
# of specific markers[2]	288	99	364
Total # of markers	412	223	488

[1] Common markers are scored and mapped in both populations.

[2] Specific markers are scored and mapped in just one of two populations.

chromosome in Population A minus the map distances from the most distal common markers to the end of the chromosome in Population B, it was estimated that one third of the 30% difference in map lengths between the populations was due to an extension of the chromosome lengths by distal markers only scored in Population A.

DISCUSSION

Polymorphism rates

As expected the polymorphism rate between the two species *L. sativa* and *L. saligna* was very high (81 %). The polymorphism rate between the two *L. saligna* parents was also quite high (38.5%). This was not really surprising because morphologically they were also quite different. For instance, line A had pinnatifid, deeply lobed leaves and line B did not have lobed leaves. The polymorphism rate between the two *L. sativa* parents was 13.4%, which is similar as in the "Calmar × Kordaat" map (Kesseli et al.1994). In consequence, our integrated map consists predominantly of markers that discriminate between *L. sativa* and *L. saligna*. In addition, it provides several markers that can be used to distinguish between *L. saligna* lines and between *L. sativa* cultivars, although the latter to a lesser extend.

Segregation distortion

The observed distorted segregation ratios calculated from the AFLP markers in the populations were only similar between the populations for the top of Chromosome 5, favouring *L. saligna* alleles. This may mean that gametes with one or more *L. saligna* alleles on the top of Chromosome 5 have a much higher fitness than those genotypes with the corresponding *L. sativa* alleles. The observed selection for heterozygotes on Chromosome 8 of Population A can be due to a locus with a high overdominance effect.

The amount and severity of observed skewness in the F_2 populations was similar to other reported skewnesses in F_2 populations, like tomato (Haanstra et al. 1999), onion (Van Heusden et al. 2000) and maize (Vuylsteke et al. 1999)

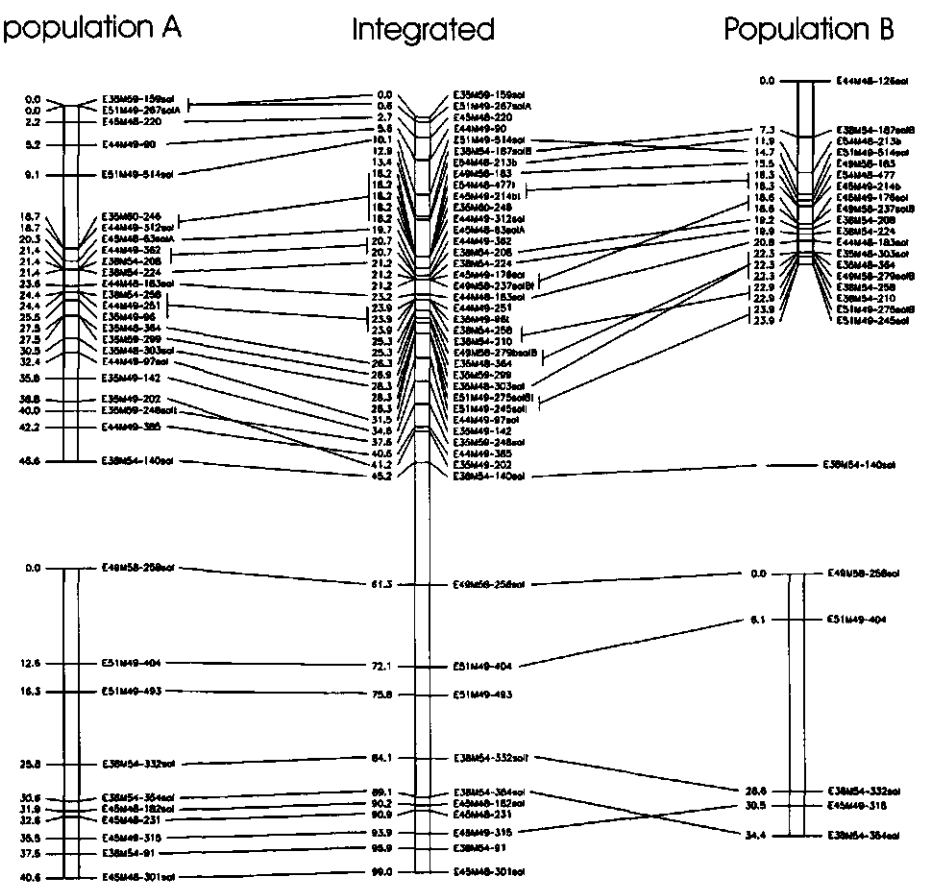


FIGURE 2. Comparison of Chromosome 8 of the integrated map and the corresponding linkage groups of Population A and B. For the nomenclature of markers see legends of Figure 1. Common markers between maps are connected by lines.

Map construction

The high level of polymorphism between *L. sativa* and *L. saligna* and the high number of loci simultaneously analysed per experiment by the AFLP technique facilitated the efficient construction of genetic linkage maps of the two interspecific populations.

When the individual maps of the populations were compared, both were highly similar in marker order and distances. The 30% difference in map length between the populations can be explained by two causes. First, map inflation is known to result from scoring errors, even if these occur at a rate below 2%. This is because errors induce an increase of recombinants. This relative map inflation becomes more severe as the average marker distance gets smaller (Lincoln & Lander 1992). So Population

A, having more markers than Population B, will for this reason result in a longer map distance than Population B.
Secondly one third of the 30% difference in map length between the populations can be explained by the presence of more distal markers in Population A compared to Population B.
The high similarity in marker order and in marker distance among the two maps facilitated the integration of the maps. The integrated map consists of nine linkage groups, has 488 markers and is 854 cM long. Compared with the "Calmar × Kordaat" map of more than 13 groups, 319 markers and 1950 cM, our map shows the expected number of chromosomes and is considerably shorter. Striking differences between the construction of the maps are: a) our integrated map used 180 (126 +54) instead of 66 F_2 plants as mapping population, b) the "Calmar × Kordaat" map contains 41 % RAPD markers which are now considered as poorly reproducible. c) different mapping software with different mapping functions was used. For our integrated map JoinMap 2.0 (Stam et al.1995) was used instead of Linkage 1 (Suiter et al. 1983) and Mapmaker 2.0 (Lander et al.1987) for the "Calmar × Kordaat" map.
In the present study AFLP markers have shown to be reliable, efficient and locus specific markers. This latter is shown by the fact that out of 125 previously considered common markers 124 were mapped on the same locus.
Codominant and monolocus SSRs are also reliable and very informative, but are less efficient as AFLP markers and therefore not recommended for generating a map. Moreover, in the present study only four SSRs could be scored codominantly. This reflects that SSRs are more informative for closely related genetic populations in lettuce.

Random distribution of markers

Several publications on genetic linkage maps with AFLP markers based on the *EcoRI*/*Mse*I restriction enzyme combination report that these markers tend to cluster around centromeric regions (Haanstra et al. 1999; Qi et al. 1998; Vuylsteke et al 1999; Young et al. 1999). An excess of repeats in the centromer may explain this phenomenon, observed in other crops. These repeats may have relatively more one-basepair-mutations detected by AFLPs and less recombination than other regions of the genome, which results in the AFLP clusters on the map.
Severe clustering of markers was not manifest in the present genetic linkage map of lettuce. If the above mentioned theory holds true, the centromeric regions of lettuce will have relatively fewer repeats compared to the rest of the genome and compared to other crops like tomato, barley, maize and soybean. Alternatively, the centromere in lettuce could be much smaller compared to the other crops. In this case the regions with suppressed recombination are much smaller.

ACKNOWLEDGEMENTS

We thank the Dutch breeding companies for the generation of the mapping populations and for their financial and advisory support. We thank R. Niks for his advisory support, P. Stam for critical reading of the manuscript and C. van de Wiel and R. Michelmore for their SSRs primers and support for the SSR analyses.

3

LACTUCA SALIGNA, A NON-HOST FOR LETTUCE DOWNY MILDEW (*BREMIA LACTUCAE*), HARBORS A NEW RACE-SPECIFIC *DM* GENE AND THREE QTLS FOR RESISTANCE

Marieke Jeuken and Pim Lindhout

Accepted for publication in Theoretical and Applied Genetics

Lactuca sativa **(lettuce) is susceptible to** ***Bremia lactucae*** **(downy mildew). In cultivated and wild** ***Lactuca*** **species,** ***Dm*** **genes have been identified that confer race-specific resistance. However, these genes were soon rendered ineffective by adaptation of the pathogen.** ***Lactuca saligna*** **(wild lettuce) is resistant to all downy mildew races and can be considered as a non-host. Therefore,** ***L. saligna*** **might be an alternative source for a more durable resistance to downy mildew in lettuce. In order to analyze this resistance, we have developed an F_2 population based on a resistant** ***L. saligna*** **× susceptible** ***L. sativa*** **cross. This F_2 population was fingerprinted with AFLP markers and tested for resistance to two** ***Bremia*** **races NL14 and NL16. The F_2 population showed a wide and continuous range of resistance levels from completely resistant to completely susceptible. By comparison of disease tests, we observed a quantitative resistance against both** ***Bremia*** **races as well as a race-specific resistance to** ***Bremia*** **race NL16 and not to NL14. QTL mapping revealed a qualitative gene (*R39*) involved in the race-specific resistance and three QTLs (*RBQ1*, *RBQ2* and *RBQ3*) involved in the quantitative resistance. The qualitative gene *R39* is a dominant gene that gives nearly complete resistance to race NL16 in** ***L. saligna*** **CGN 5271 and therefore it showed features similar to** ***Dm*** **genes. The three QTLs explained 51% of the quantitative resistance against NL14, which indicated that probably only the major QTLs have been detected in this F_2 population. The perspectives for breeding for durable resistance are discussed.**

Several *Lactuca* species are host for the biotrophic oomycete *Bremia lactucae* (downy mildew; Lebeda and Syrovatko 1988). Because of major yield losses in lettuce

(*Lactuca sativa*) cultivation due to downy mildew, lettuce breeders have put a large effort into obtaining resistance to this pathogen. In most lettuce cultivars *Dm* genes confer race-specific resistance to downy mildew (*Bremia lactucae*). The resistance of these *Dm* genes is controlled by single dominant genes that are matched by avirulence genes in *Bremia* in a gene-for-gene interaction, i.e. race-specificity. This results in an incompatible interaction associated with a hypersensitive response of the host (Crute & Johnson 1976). During the history of lettuce breeding 19 *Dm* genes have been identified and have been introgressed into commercial cultivars from cultivated germplasm sources or closely related species like *L. serriola* (Landry et al.1987, Crute 1992, Bonnier et al. 1994, van Ettekoven and van der Arend 1999). The resistance of *Dm* genes is not durable since these genes become ineffective soon after their introduction as a result of rapid genetic adaptation of the pathogen (Crute 1992, Reinink 1999, Lebeda and Schwinn 1994). Two parameters play an important role in this high evolutionary potential of *Bremia* populations: 1) A mixed reproduction system (asexual and sexual), which can cause fast fixation of new virulence alleles or allele combinations arising from recombination and mutation (Crute 1992, McDonald and Linde 2002). 2) New isolates are rapidly spread (=high gene flow) due to wind dispersal of spores. Since race-specific *Dm* genes are not durable, there is a need for an alternative, race non-specific and durable resistance in lettuce breeding.

In search for an alternative resistance, attempts have been made to exploit partial resistance in butterhead lettuce (Eenink 1981, Eenink et al. 1982, Eenink and De Jong 1982, Eenink et al 1983). However, these attempts have not resulted in commercial breeding and the release of cultivars, specifically bred for increased level of partial resistance (Reinink 1999).

In addition to screening for resistance within the *L. sativa* species, the biodiversity for *Bremia* resistance has been surveyed in species closely related to *L. sativa*. This survey of four *Lactuca* species suggested that, of the *Lactuca* species that can be crossed with cultivated lettuce, only *L. saligna* (52 accessions tested) is completely resistant to all *Bremia* races (20 races tested) and may be considered a non-host (Bonnier et al.1992) This non-host status for *L. saligna* was already suggested earlier in small-scale experiments (Norwood 1981, Gustafsson 1989, Lebeda and Boukema 1991). At the histological level the *L. saligna* accessions varied in resistance symptoms as presence or absence of necrosis formation after *Bremia* inoculation (Lebeda and Reinink 1994). *L. saligna* accessions with *Bremia* resistance without necrosis formation are a very interesting source for alternative resistances.

Very little is known about the genetics of resistance in non-host species (Heath 2001, Kamoun 2001). It remains unclear whether the phenomenon "non-host resistance"comprises one or several defense mechanisms explained by known or a new types of resistance. Therefore, a study on the resistance of *L. saligna* to *Bremia* may reveal new insights into the "non-host" defense mechanisms of plants. In this study we investigated the genetics and specificity of *Bremia* resistance in *L. saligna*.

MATERIALS AND METHODS

Plant material and genotype (linkage) analysis

An F_2 population was generated from the cross of *L. saligna* CGN 5271 as resistant female parent with *L. sativa* cv "Olof", a butterhead cultivar as male parent. Olof is considered to be a generally susceptible cultivar for all European *Bremia* races and devoid of effective *Dm* genes. The F_2 population was derived from a single F_1 plant and consisted of 126 individuals. Plant material and DNA samples were prepared as described in Jeuken et al. (2001).
All 126 F_2 plants have been fingerprinted by AFLP analysis for the construction of a genetic linkage map. This map consisted of 488 markers on nine linkage groups covering 854 cM (Jeuken et al. 2001). All F_2 plants were selfed and the F_3 seed was collected. F_3 plants from selected lines were grown in a randomized block design.

Bremia disease tests

F_2 population

Bremia was maintained in plastic boxes on seedlings of susceptible cultivars. The inoculum for disease tests was prepared by washing sporulating seedlings in tap water. The spore concentration was adjusted to 3×10^5 spores per ml.
Disease tests were performed on mature plants with two *Bremia* races. Per *Bremia* race two tests were done on leaf discs of nine and ten weeks old plants (i.e. first and second test). Per disease test four leaf discs of 17 mm in diameter were taken from full-grown leaves of each F_2 plant and placed upside down on filter paper moistened with water in a plastic box of 40×25×8 cm. The four leaf discs of each F_2 plant were placed per pair randomly in two replicates in the box. As controls at least six leaf discs of the susceptible parent *L. sativa* Olof and at least four leaf discs of the resistance parent *L. saligna* CGN 5271 were included in each box, which contained in total 198 leaf discs. Transparent plastic lids covered the boxes. Growth conditions were a photoactive period of 16 hours and a constant temperature of 15° C. The leaf discs were inoculated by spraying with a spore suspension. To minimize the risk of escapes, a second inoculation was performed the day after the first inoculation. After inoculation the leaf discs were incubated in the dark for 12 hours. Ten days after inoculation, the leaf discs were assessed for sporulation. Leaf discs were scored for infection severity according the following classes: class 0: no sporulation, class 1: 1-25% of leaf disc area sporulates; class 2: 26-50%; class 3: 50-75% and class 4: 75-100% (Lebeda and Pink 1998, Lebeda and Reinink 1991). Per disease test the infection severity score of an F_2 plant was calculated as the average of the observations of the four leaf discs. A low or high infection severity was interpreted as resistance or susceptibility, respectively. Per disease test, an analysis of variance was carried out to calculate environmental variance and the wide sense heritability (H^2). $H^2 = S^2_g / S^2_g + S^2_e$, in which S^2_g is the variance between genotypes and S^2_e is the variance within genotypes based on four observations per genotype.

F_3 lines

A disease test with *Bremia* race NL16 was performed on eight F_3 lines on mature plants of eleven weeks old. The *Bremia* disease test was performed as described above, except that from each F_3 plant six leaf discs were taken and placed per pair in three replications randomly in the box. Furthermore, *Bremia* infection severity was scored on leaf discs distributed as the percentage of leaf area covered with sporulation, instead of scoring the infection severity in classes. To make comparisons with the F_2 disease test results, the plant and line averages of the F_3 observations were transformed to infection severity class units.

QTL mapping

The software program MapQTL 4.0 (Van Ooijen and Maliepaard, 1996) was used to perform the Kruskal-Wallis test, the Interval Mapping method and the Restricted MQM Mapping method on each data set of the four *Bremia* disease tests of the F_2 population. The Kruskal-Wallis test is a nonparametric test in which no assumptions are being made for the probability

distributions of the quantitative trait (after fitting the QTL genotype). In Interval Mapping and MQM Mapping distributions are assumed to be normal. The complete DNA marker data set of 488 markers was used in the Kruskal-Wallis test and the Interval Mapping. A subset of 182 markers, spaced approximately 5 to 15 cM, was used as a framework map for the restricted MQM Mapping method. These markers were chosen on the basis of maximal information content and genome coverage.
A data set of a *Bremia* disease test consisted of the average infection severity score per F_2 plant based on observations on four leaf discs.
The criterion for detecting a QTL was set by a significance level of 0.005 in the Kruskal–Wallis test. For the Restricted MQM method a LOD threshold of 3.7 was used (Van Ooijen, 1999).
In this paper the QTL mapping results are presented on the F_2 population with two adjusted scores, which were based on disease test results of corresponding F_3 lines.

RESULTS

Bremia disease tests on the F_2 population

The two *Bremia* races NL14 and NL16 were used in disease tests. NL16 was chosen since it was the most common and complex race on lettuce in Europe. Race NL14 was chosen as a contrasting race. Race NL14 and NL16 are virulent on cultivars with *Dm1*, *Dm2*, *Dm3*, *Dm4*, *Dm5/8*, *Dm6*, *Dm10*, *Dm11*, *Dm13*, *Dm14* and *Dm16* (Table 1). NL14 and NL16 have no avirulence factors that interact with *Dm15*, *R17*, *R18*, *R36*, *R37* and *R38* (according to convention for *Bremia* resistance nomenclature; Van Ettekoven and Van der Arend 1999).

TABLE 1. Differential set of lettuce genotypes used to characterize two isolates of *Bremia lactucae* for virulence spectrum. "+" indicates a compatible/ susceptible reaction. "–" indicates an incompatible/ resistant reaction. [a] These lines were used as controls during maintenance and propagation of *Bremia* races.

Cultivar / line	*Dm* gene/ *R*-factor	*Bremia* NL14	*Bremia* NL16
Olof	none	+	+
Lednicky	1	+	+
UcDm2	2	+	+
Dandy	3	+	+
R4T57	4	+	+
Valmaine	5/8	+	+
Sabine	6	+	+
Reskia	3 and 7	-	+
UCDm10	10	+	+
Fila	2 and 11	+	+
Norden	3 and 11	+	+
Pennlake	13	+	+
UcDm14[a]	14	+	-
Spiky[a]	14	+	-
PIVT1309	15	-	-
Strada[a]	16	-	+
Luxor[a]	2 and 16	-	+
Mariska	18	-	-

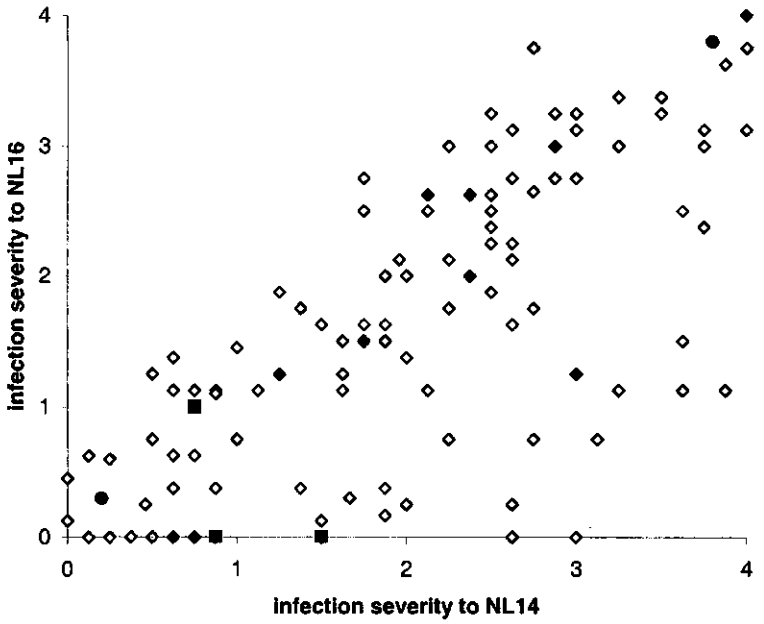


FIGURE 1. Comparison of the F_2 disease tests against *Bremia* races NL14 and NL16. The infection severity scores (diamond and square symbols) are expressed as the average class number per F_2 plant based on observations on maximally eight leaf discs in two experiments. *L. sativa* Olof is the susceptible control (27 and 36 observations for NL14 and NL16) and *L. saligna* CGN 5271 is the resistant control (17 and 24 observations; black dot symbols). Infection severity scores: class 0: no sporulation, class 1: 1-25% of leaf disc area sporulates, class 2: 26-50%, class 3: 50-75% and class 4: 75-100%. The infection severity scores represented by two and three F_2 plants are shown by black diamonds and black squares.

The F_2 population showed a wide and continuous range in infection severity scores from completely uninfected (scale value 0) to completely infected (scale value 4) in tests with both *Bremia* races. The susceptible parent *L. sativa* Olof showed an average infection severity score of 3.8 against NL14 and 3.8 against NL16. The resistant parent *L. saligna* CGN 5271 showed an average infection severity score of 0.2 against NL14 and 0.3 against NL16. If sporulation occurred on the *L. saligna* parent, it was only seen at the cutting edge of the leaf disc. The score on this resistant reference accession never increased more than 5% of the leaf area (class 1 on the disease class scale).

The heritability was 0.86 in the first test and 0.80 for the second test with NL16 and in the two tests with NL14 0.82 and 0.74 respectively. These high heritabilities imply a high genetic variation and a small error in these disease tests on this F_2 population.

The Pearson correlation coefficient between two tests with the same race was 0.75 and 0.80 for NL14 and NL16 respectively. Similar results appeared for tests with two *Bremia* races. To present the results of the tests with different *Bremia* races, we averaged the infection severity scores of F_2 plants from two tests with the same race. In this way, the number of missing values of plant and/or leaf discs observations was minimized. Because of the high correlation between disease tests with the same race, it was not necessary to make corrections to standardize the two tests before averaging. The results of the tests with different *Bremia* races were compared for the detection of a possible race-specificity of the resistance (Figure 1). Two trends were observed in this comparison. Firstly, the largest group of plants was scattered around the diagonal, indicating that their resistance level against NL14 and NL16 was very similar. Secondly, a small group of plants had a high NL16 resistance and low NL14 resistance, but an opposite group with a low resistance to NL16 and a high resistance to NL14 was not present. These results suggested mainly race non-specific effects, however also some plants with specific resistance to NL16.

QTL mapping

As a wide and continuous range of infection severity scores in F_2 plants was observed, the conclusion was drawn that the resistance was (at least partly) a quantitative trait. To dissect and map the underlying genes, we performed QTL mapping on each data set of each disease test separately. This revealed four QTLs in the F_2 population. The disease test results of the F_2 population corresponded well with the detected QTLs, except for five F_2 plants. Their disease test results seemed not to agree well with the average disease test result of all F_2 plants with the same genotype at the specific locus of a detected QTL. To check whether the result of the disease test or the genotyping result for this F_2 plant was incorrect, we tested the F_3 line in a disease test with race NL16. F_3 seeds were available from three out of five F_2 plants. An ambiguous infection severity score of the F_2 parent was adjusted by the average of infection severity scores of the resistant F_3 plants if there was a difference of one unit (1.0) or more on the infection severity class scale (0-4) between the F_2 and the F_3 data. Disease test results of two out of three F_2 plants (38 and 61) have been adjusted based on disease test results of corresponding F_3 lines (Table 3).

In general, the detected QTLs were identified as being significant in both tests with the same *Bremia* race. However, sometimes a QTL just below the significance level was observed, except for one QTL (*RBQ1*), which was not detected in the second disease test with race NL16(on ten weeks old plants).

The resistance against *Bremia* NL16 was mainly explained by one QTL on the top of Chromosome 9 with a LOD score of 18 and an explained variance of 54% (Table 2 and Figure 2). This QTL was not identified with the *Bremia* disease test data sets of race NL14.

The top of Chromosome 9 has a distorted segregation ratio with an excess of *L. sativa* alleles (Jeuken et al. 2001). At the QTL locus, 71 plants were homozygous for the *L. sativa* alleles, 47 plants were heterozygous and no plants were homozygous for the

TABLE 2. Detected QTLs for *Bremia* resistance in an F_2 population from *L. saligna* × *L. sativa*. QTLs were identified if the threshold of the Kruskal-Wallis test or the Restricted MQM mapping was exceeded. QTLs are presented per *Bremia* race from the disease test with the highest LOD score.

QTL name	Chromosome number and QTL interval [f]	*Bremia* race	Kruskal - Wallis test [a] significance	Restricted MQM mapping Fit dominance yes [b]		
				Peak LOD Score[d]	Exp%	Add
R39	9, 3 cM	NL16	xxxx	18[c]	54	2.1
RBQ1	7, 59-65 cM	NL16	xxx[e]	3.8[e]	24[e]	0.9[e]
		NL14	xxxx	7.5	26	0.7
RBQ2	1, 15-21 cM	NL16	x	*3.6*[c]	12	0.5
		NL14	x	*3.1*	13	0.5
RBQ3	9, 32-41 cM	NL16	xxx	5.3	23	0.5
		NL14	xx	3.9[c]	12	0.3

a The Kruskal-Wallis test P- value = significance level
x = 0.005, threshold significance level
xx = 0.001
xxx = 0.0005
xxxx = 0.0001

b Exp% = proportion of the explained phenotypic variance.
Add = additive effect of the resistance allele.

c = use of cofactors at LOD peaks
d = italic numbers represent LOD values below threshold
e = These data were obtained by QTL mapping on 71 F_2 plants with susceptibility alleles for *R39*
f = This QTL interval covers a chromosome region harboring markers that show a Kruskal Wallis test P-value > 0.005.

L. saligna alleles. For eight plants it was not possible to distinguish whether they were heterozygous or homozygous for *L. saligna* alleles because the informative AFLP marker, linked to the QTL, was not scored codominantly. The plants, that were homozygous for the *L. sativa* alleles of the QTL locus, had an average infection severity score of 3.0 (based on first NL16 disease test data), while the plants heterozygous at this locus showed an average infection severity score of 0.9 (based on first NL16 disease test data). The effect of one allele of this gene was therefore high as it decreased infection severity by 2.1 units on the infection severity scale. This large effect and its race-specific nature, suggested that this "QTL" represents a qualitative race-specific resistance gene similar to *Dm* genes. At the locus of this gene no *Bremia* resistance gene has been mapped before (see Discussion). We propose to designate this new gene *R39*, which follows the previously cited *R38* gene (van Ettekoven and van der Arend 1999).

The three other QTLs had smaller effects on the infection severity level against both races and were designated *RBQ1*, *RBQ2* and *RBQ3* (*Resistance to Bremia QTL*)(Table 2). *RBQ1* gave resistance against NL14 and also showed resistance against NL16 with a LOD value of 3.6 just below the threshold (3.7). Because *R39* explained a large part of the resistance to NL16, we selected 71 F_2 plants with susceptible alleles for *R39* and repeated QTL mapping. Now *RBQ1* was identified as being significant (Table 2).

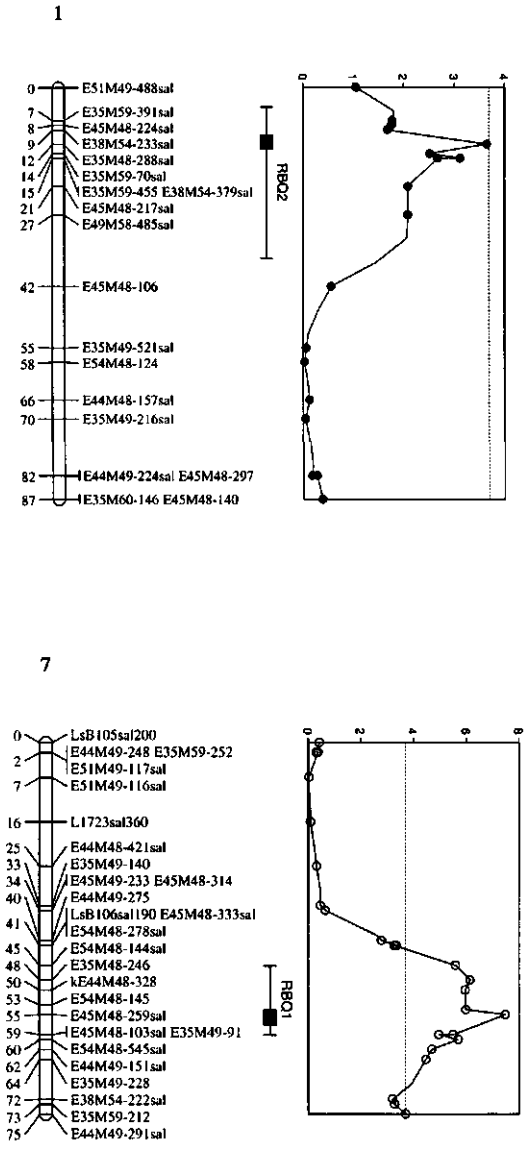


FIGURE 2. The position of QTLs for *Bremia* resistance are shown beside the marker linkage maps of chromosome 1, 7 and 9, which are derived from the F_2 mapping population *L. saligna* × *L. sativa* (Jeuken et al 2001). Map positions are given in cM. Bars indicate the QTL interval in which the inner bar shows a one LOD support confidence interval and the outer bar shows a two LOD support confidence interval. Graphs near the chromosomes show the QTL likelihood profile based on restricted MQM mapping with the use of cofactors. Lines with solid circles are based on results of a disease test with *Bremia* race NL16. Lines with open circles are based on results of a disease test with *Bremia* race NL14. The LOD threshold value of 3.7 is shown as a dotted line.

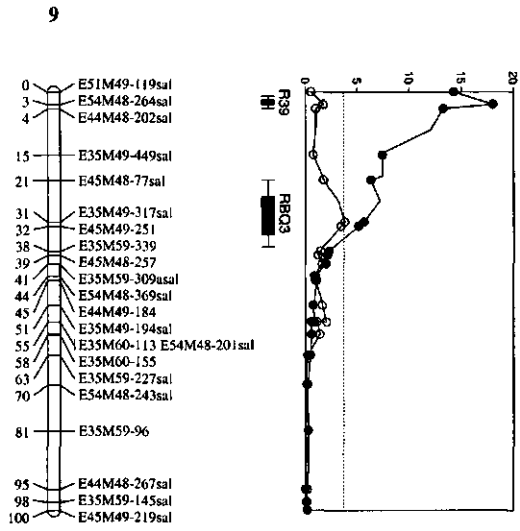


FIGURE 2. Continued

RBQ2 was identified against NL14 and NL16 with the Kruskal-Wallis test, but its LOD score in the Restricted MQM Mapping was just below the significance level. *RBQ3* was positioned approximately 28 cM away from the *R39* locus. This made it difficult in the NL16 data sets to distinguish it from *R39* and to define it as a separate QTL. An extra peak in the LOD profile was observed with a LOD score of 5 (Figure 2).

Verification of QTLs by five F_3 lines

We performed a disease test on F_3 lines to verify QTLs detected in the F_2 population. Therefore, we used marker assisted selection to select for F_2 plants with none, one or two QTLs preferably in homozygous state. The resistance alleles of a QTL were considered to be present if all scored markers in the QTL interval (Table 2) supported the homozygous *L. saligna* state of that locus. Similarly, susceptibility alleles were selected by markers in homozygous *L. sativa* state in this interval. In total five F_3 lines consisting of at least seven plants, were used for *Bremia* disease test with race NL16 for QTL verification. Four F_3 lines were selected for presence of QTLs based on flanking markers and they indeed showed resistance levels (Table 3, Figure 3b). Segregation of resistance was observed in F_3 lines from F_2 plants with a heterozygous QTL like plant 34 and 90. Remarkably, F_3 line 35 was selected for susceptibility and lack of QTLs, but still three out of seven plants were intermediate resistant with an infection severity score of 1 to 2.

In conclusion, resistant plants can be selected on the genotype level by marker assisted selection.

TABLE 3. The infection severity scores of F_2 parents and F_3 lines based on the QTL allele composition. F_3 line infection severity scores are estimated from maximally 72 observations (12 plants × 6 observations) and the F_2 parent resistance value is estimated from maximally eight observations (1 plant × 4 observations × 2 tests). QTL genotype: a = homozygous *L. sativa*, h = heterozygous, b = homozygous *L. saligna*, c = b or h, d = a or h not distinguishable with dominant markers, u = unknown. * = average based on resistant plants only.

F_2 plant number, F_3 line number	QTL genotype of F_2 parent				Average infection severity score against *Bremia* NL16			
	R39	***RBQ1***	***RBQ2***	***RBQ3***	Expected in F_2 plant based on additive effect (Table 2)	Observed in F_2 parent and expected for F_3 line	Observed in F_3 line	# of tested F_3 plants
Experiment to check F_2 plants with ambiguous disease results								
61	h	a	a	a	3.8–2.1= 1.7[e]	1.5	0*	11
123	c	a	a	h	3.8–2.1= 1.7[e]	3.7	3.5	12
38	h	a	u	h	3.8–2.1= 1.7[e]	2.5	0.4*	12
Experiment for verification of QTLs								
113	a		b	d	3.8–(2*0.9)-(2*0.5)= 1.0 or d= h then 1.0–0.5= 0.5	1.0	0.5	12
34	a	a	h	a	3.8–0.5= 3.3	2.0	1.8	11
90	a	a	h	a	3.8–0.5= 3.3	3.3	2.7	12
35	a	a	a	a	3.8	4.0	2.4	7
107	a	a	b	d	3.8–(2*0.5)= 2.8 or d=h then 2.8–0.5= 2.3	1.6	0.7	11
Controls						F_2 disease tests	F_3 disease test	
L. sativa	a	a	a	a		3.8	3.9	
L. saligna	b	b	b	b		0.3	0.2	

e = the average resistance level of 47 plants with one *R39* allele was 0.9 and for 71 plants with no *R39* allele it was 3.0. Therefore, 0.9 would be a more realistic expected resistance level than 1.7.

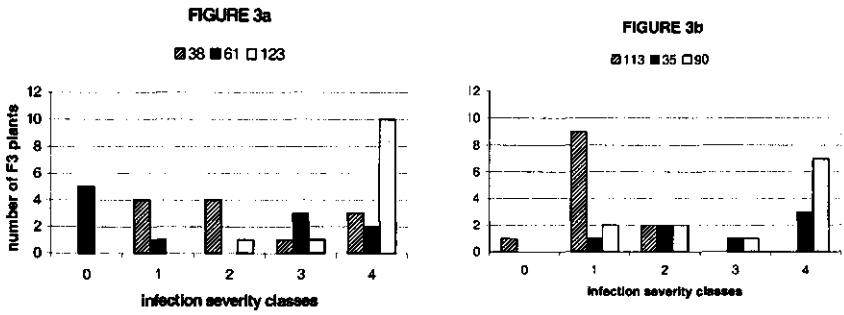


FIGURE 3. Frequency distribution of F_3 lines according to infection severity classes against *Bremia* NL16. Plants, scored in classes from 0 to 4, are interpreted as in a range from completely resistant to completely susceptible plants. For explanation see legends Table 3.

DISCUSSION

Disease tests on F_2 population

Based on the fact that the variance in observations on the four leaf discs per F_2 plant was low and the heritability high, we conclude that the disease tests were accurate. Furthermore, the majority of the disease test results of the F_3 lines were in agreement with the F_2 parent infection severity scores, indicating a good repeatability. With these four data sets of infection severity scores of F_2 plants, the same QTLs were found in all performed tests (of MapQTL 4.0), which confirms the reliable detection of QTLs.

A new race-specific resistance gene *R39*

From QTL mapping on data sets of disease tests with NL16, it was shown that *R39* on Chromosome 9 explained most of the resistance. In the F_2 population one allele of *R39* had already a very large effect on the infection severity score of 2.1 units. In F_3 line 61 one allele of *R39* even explained an average infection severity difference of 3.0 units between the groups of susceptible and resistant F_3 plants. This almost equals the difference between *L. sativa* Olof and *L. saligna*. The large effect and the race-specificity of *R39* suggests that it is a qualitative gene (*Dm* gene), which interacts with *Bremia* in a gene-for-gene relationship based on the hypersensitive response. Evidence for the hypersensitive response was found in a histological study in which *L. saligna* 5271 showed necroses upon inoculation with NL16 (Sedlarova et al. 1999). Resistance to race NL16 and susceptibility for NL14 is only observed for the known resistance gene *Dm*14 (Table1). However, *R39* is probably not *Dm*14 because in another histological study it was shown that the resistance of *L. saligna* 5271 (harbouring *R39*) against race NL5 (Avirulent on *Dm*14) was without necrosis (Lebeda and Reinink 1994). In addition, *Dm*14 was mapped by using the Calmar × Kordaat map on Chromosome 2 in a resistance cluster with *Dm*3 (Kesseli et al.1994). This was not consistent with the map position of *R39* on Chromosome 9 at 3 cM. *R39* was also not mapped on the positions of three other known *Dm* gene resistance clusters (Kesseli et al.1994). More information on the resistance spectrum of *R39* will be obtained by disease tests with a differential set of *Bremia* races.

The result of a *Dm*-like gene was not completely unexpected as in histological studies the observation of necroses formation on *L. saligna* accessions after *Bremia* inoculation could have been a consequence of a hypersensitive response of a potential *Dm* gene (Lebeda and Reinink 1994). Associations between hypersensitive responses and non-host resistances were seen for other oomycetes like *Phytophthora infestans* (Vleeshouwers et al. 2000) and other pathogens like *Cladosporium fulvum* (Laugé et al. 2000). Though even if *R39* is associated with the hypersensitive response, this does not proof that *R39* contributes to the non-host resistance of *L. saligna*. Also it is not clear why *Bremia*, after the speciation of a common *Lactuca* ancestor into the *L. serriola/L. sativa* cluster and the *L. saligna* cluster, still contained virulence genes that could break through *R39* in *L. saligna*, while *L. saligna* is a non-host for *Bremia*.

Three QTLs for *Bremia* resistance

The three detected QTLs (*RBQ1, RBQ2* and *RBQ3*) all were effective against both races. The detection of these QTLs from tests with race NL16 was sometimes less sensitive compared to tests with NL14. This was due to the nearly complete resistance of *R39* to NL16, which in fact reduced the population size for QTL mapping to the number of F_2 plants without *R39*. However, *RBQ2* and *RBQ3* were still detected using data of all 126 F_2 plants. *RBQ1* was detected when QTL mapping was performed on all the 71 F_2 plants that were homozygous for the susceptibility alleles of *R39*.

From the three identified QTLs, *RBQ1* had the largest effect. The three QTLs together explained 51% of the quantitative resistance to NL14. As the calculated environmental variance was low this meant that probably not all QTLs for resistance have been detected yet. The major part of the unexplained variance may be due to minor QTLs that did not reach the detection level or too strong epistatic interactions, which cannot be detected by QTL mapping methods used. Another factor, which may have hampered the detection of QTLs, could have been the presence of local extreme distorted segregation ratios against wild parent alleles in the F_2 population. The possibility of undetected QTLs in the F_2 population was supported by the results of the expected susceptible F_3 line 35, in which some plants occurred with unexpectedly low infection severity scores.

We assumed that this quantitative resistance, which implied non-race specificity and was explained partly by these three QTLs, could be the main reason for the non-host status of *L. saligna* CGN 5271.

GENERAL CONCLUSION

We have been able to perform a first molecular genetical analysis of the resistance of *L. saligna* to *Bremia*. It was concluded that the dissection of the resistance has been successful as four loci were detected that harbor genes for *Bremia* resistance. This study revealed that the resistance of *L. saligna* seemed to be explained by a combination of a qualitative race-specific resistance and a quantitative resistance, which was a race non-specific, proposed non-host resistance. From breeders' perspective the localization of a quantitative resistance was exiting, as it seemed an alternative resistance to *Bremia* that is non-race specific and more durable than *Dm* genes. Molecular markers flanking the QTLs will facilitate the breeding for this quantitative resistance.

ACKNOWLEDGEMENT

We thank P. Stam and R.E. Niks for critically reading the manuscript and their advisory support in this study. We thank Nickerson Zwaen BV for providing the F_2 seeds.

4

DEVELOPMENT OF A SET OF BACKCROSS INBRED LINES WITH CHROMOSOME SEGMENTS OF *L. SALIGNA* (WILD LETTUCE) INTROGRESSED INTO *L. SATIVA* (LETTUCE)

Marieke Jeuken and Pim Lindhout

Backcross Inbred Lines (BILs) were developed in which chromosome segments of *L. saligna* (wild lettuce) were introgressed into *L. sativa* (lettuce). These lines were developed by four to five backcrosses and one generation of selfing. The first three generations of backcrossing were randomly. Marker Assisted Selection was started in the BC_4 generation. A set of 29 lines was selected that together contained 95 percent of the *L. saligna* genome. Of these lines, 16 had a single homozygous introgression (BILs), one had two homozygous introgressions, five lines had heterozygous single introgressions and seven lines had two or more heterozygous introgressions. Segregation ratio's in backcross generations were compared to distorted segregation ratios in an F_2 population, and indicated that most distorted segregations can be explained by genetic effects on pollen- or egg cell fitness. By BIL association mapping two morphological traits viz. 'pointed leaf apex' and 'reflexed involucre' from *L. saligna* and several additional AFLP markers were mapped.

When genetic studies are performed to unravel the genes behind traits of plants, we can discern discrete traits, often explained by single genes in Mendelian patterns of inheritance and quantitative traits, explained by quantitative trait loci (QTLs) with complex patterns of inheritance. The genetic analysis of quantitative traits requires large segregating populations with good fertility, vigour, similar plant architecture and physiology and limited distorted segregation (Lander and Botstein 1989). Several genes of quantitative traits in crops have been mapped by using QTL mapping procedures (e.g.Young 1996, Grandillo et al 1999). If segregating populations do not meet the prerequisites that allow QTL mapping, advanced backcross lines like

Backcross Inbred Lines (BILs) can be an interesting alternative. BILs are lines obtained by several generations of backcrossing with one of the parents (=recurrent parent) starting from the F_1 and at least one final generation of selfing. These lines have a high percentage (mostly >90%) of recurrent parent genome and a low percentage (mostly <10%) of wild parent genome. Intentionally, each BIL contains one introgression segment and a complete set of BILs should cover the complete genome of the wild species. For the purpose of genetic analysis, a complete set of BILs has several advantages over other types of segregating populations: 1) The high genetic and morphological similarity between lines enables more precise estimates of quantitative traits. 2) BILs are homozygous lines, which allows infinite replication of measurements and experiments, also in different seasons and environments. In this way, specific QTL × environment interactions can be studied. 3) The interaction between several QTLs can be studied by intercrossing of the BILs harboring the respective introgression segments. 4) A practical advantage of BILs for commercial breeding purposes is that due to the low percentage of wild parent genome, the introduction of an interesting trait into a commercial cultivar will be relatively straightforward and rapid.

Several sets of BILs have already been developed for tomato and their wild relatives like *Lycopersicon pennellii* in *L. esculentum* (Eshed and Zamir 1994), *L. hirsutum* in *L. esculentum* (Monforte and Tanksley 2000) and *Solanum lycopersicoides* in *L. esculentum* (Chetelat and Meglic 2000). In rice, a set of BILs was made between a *japonica* and an *indica* variety (Lin et al. 1996, Lin et al. 1998). These sets of BILs were all developed with the use of Marker Assisted Selection (MAS), but the number of backcrosses and selfings varied. For example, BILs have been derived from $BC_1S_6BC_3S_1$ (Eshed and Zamir 1994), BC_2S_3 (Monforte and Tanksley 2000), BC_2S_6 (Chetelat and Meglic 2000) and BC_1S_5 (Lin et al. 1998). Several studies on these sets of BILs have already shown their usefulness for mapping and characterizing genes and/or QTLs (Eshed and Zamir 1995, Lin et al. 1988, Monforte and Tanksley 2001, Zamir 2001).

In the present paper we report on the development of a set of lettuce BILs with introgressions from the wild lettuce species *Lactuca saligna*. *L. saligna* is an interesting resource of resistance to lettuce downy mildew (*Bremia lactucae*). A survey on the biodiversity for *Bremia* resistance on several *Lactuca* species (including mainly *L. sativa*, *L. serriola*, *L. virosa* and *L. saligna*) suggested that only *L. saligna* is completely resistant to all *Bremia* races and can be considered a non-host (Norwood 1981, Gustafsson 1989, Lebeda and Boukema 1991, Bonnier et al.1992). Despite many efforts, breeders have not been able to introduce this resistance into cultivated lettuce. We have made an effort to map this *Bremia* resistance by screening an F_2 population from a *L. saligna* × *L. sativa* cross (Jeuken et al. 2001). This F_2 population was not optimal for mapping QTLs that were involved in *Bremia* resistance. This was due to: 1) Limited population size due to reduced germination and vigour (23% of the F_2 seeds did not result in adult F_2 plants); 2) Extreme variation in plant architecture and development among F_2 plants; 3) Severe distorted

segregations for several chromosome regions; 4) Sterility (37% of the F_2 plants were sterile), preventing unbiased F_3 testing. Still, some QTLs were identified. However, in view of the above-mentioned limitations we expected that not all QTLs for resistance to *Bremia* from *L. saligna* were mapped in this F_2 population. Therefore, we developed a set of BILs with chromosome segments of *L. saligna* introgressed into *L. sativa*.

A precise selection tool is crucial for developing a set of BILs. For a fast selection process and for assessment of the degree of coverage of the genome of the wild parent, a saturated and reliable genetic map consisting of equally dispersed high-throughput DNA markers is required. However, at the start of our backcross program neither such markers nor a genetic map was available and we chose to develop the first three backcross generations randomly without Marker Assisted Selection. Meanwhile, an interspecific AFLP linkage map was developed based on an F_2 population (Jeuken et al. 2001). This genetic map was immediately used for Marker Assisted Selection in the fourth backcross generation (BC_4).

In this study, we report the development of a set of Backcross Inbred Lines (BILs) with introgressions of *L. saligna* in a *L. sativa* background. Our goal is to achieve a set of BILs that covers the complete genome of *L. saligna* while each BIL contains only one homozygous introgression of *L. saligna*.

MATERIALS AND METHODS

Plant materials and development of Backcross Inbred Lines

The same cross of *L. saligna* CGN 5271 × *L. sativa* cv. Olof was used for this backcross program as was used for the development of an F_2 population to construct a genetic linkage map (Jeuken et al. 2001). A single F_1 plant was backcrossed with *L. sativa* as a father (Figure 1). The BC_1 generation was crossed reciprocally with *L. sativa* Olof to obtain the BC_2. BC_2 and BC_3 plants were backcrossed with *L. sativa* Olof as a mother to obtain the BC_3 and BC_4 respectively. From the BC_4 generation we analyzed 84 individuals with 267 AFLP markers. According to the genetic map of *L. saligna* × *L. sativa* (Jeuken et al. 2001), graphical genotypes were obtained from each BC_4 plant using the software program Graphical GenoTyping (GGT, Van Berloo 1999, http://www.dpw.wau.nl/pv/pub/ggt/). Based on the graphical genotypes, plants with one introgression of *L. saligna* were selected for selfing and plants with two or more introgressions were selected for further backcrossing. For those regions of *L. saligna* that were lacking in this BC_4, more AFLP analyses were made on several plants of the BC_3, the BC_2 and all plants of the BC_1 generation to identify genotypes that contained one or more of the lacking chromosome fragments. Further backcrossing and selection on these plants was performed similarly as on the BC_4 lines described above.

Incidentally, phenotypic selection preceded the AFLP genotyping, when a clear deviating phenotype was associated with a particular introgression fragment. At the final stage of BIL development, progeny of the selected selfed plants with one introgression were genotyped with AFLP markers and examined for a homozygous introgression. The progeny of a plant with a homozygous introgression was designated a Backcross Inbred Line (BIL).

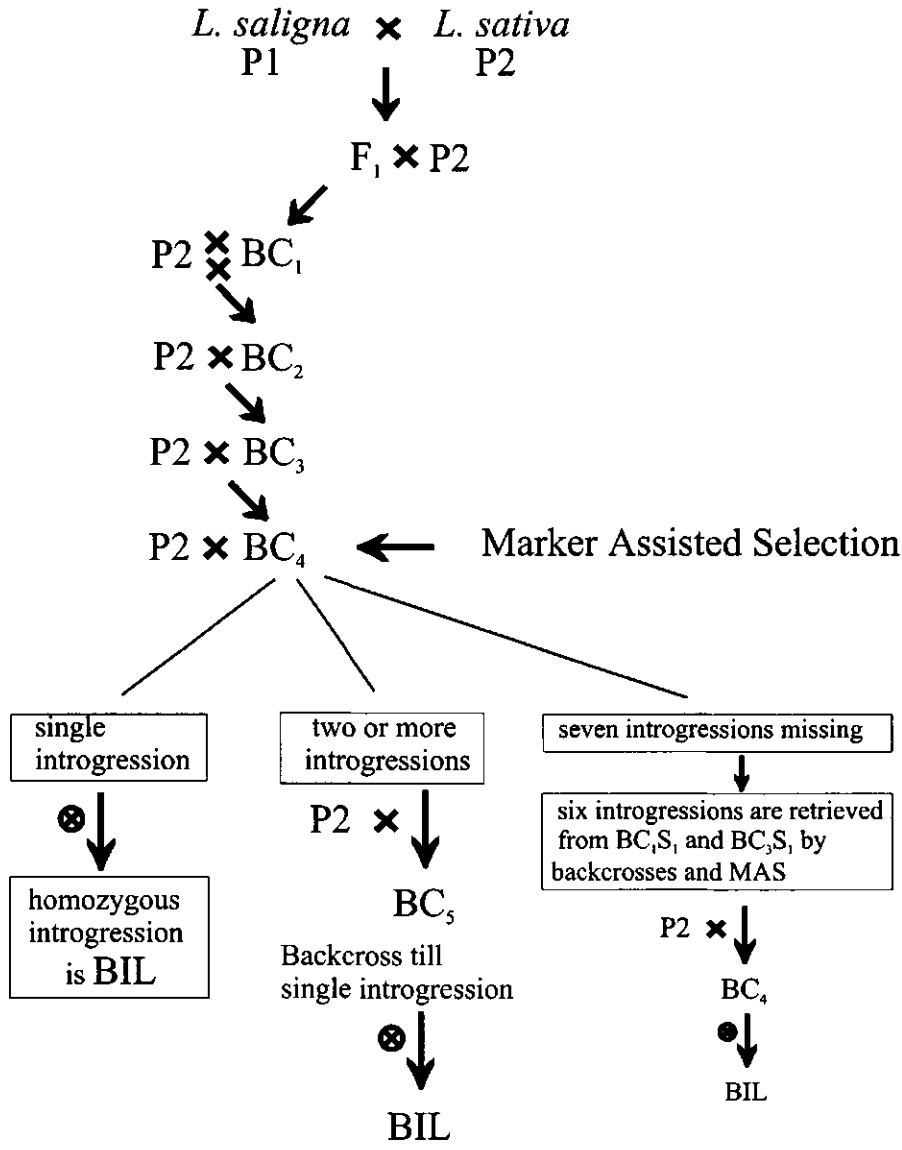


FIGURE 1. Backcross and selection program for the development of a set of BILs.

AFLP analysis

Leaf material was collected from four to eight weeks old plants. Genomic DNA was extracted from frozen leaves according to the procedure as described by Van der Beek et al. (1992) with some minor modifications: the DNA was washed overnight in 76 % ethanol and 10 mM NH_4Ac, dried and dissolved in 100 µl sterile TE buffer (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA). AFLP analyses were performed either with the radioactive label P^{33} (Jeuken et al. 2001) or with a *LI-COR* detection system using fluorchrome labeled primers. AFLP analysis with the *LI-COR* system was based on the AFLP reactions by a two step amplification described by Vos et al. (1995) with some modifications: Genomic DNA (250 ng) was digested with restriction enzymes and simultaneously ligated with adapters. The preamplification was performed in a volume of 20 µl by a PCR of 24 cycles with the following profile: 30 s denaturation at 94°C, 30 s annealing at 56°C and 60 s extension at 72°C. For the second PCR amplification we used *Eco*RI primers labeled with infrared dye IRDye 700 or IRDye 800 (*LI-COR*, Lincoln, NE, USA). Five µl of the diluted secondary template was mixed with 50 ng unlabeled *Mse*I-primer, 0.5 pmol IRD700-labeled *Eco*RI-primer or 0.6 IRD800-labeled *Eco*RI-primer in 0.2 mM of all four dNTPs and 0.2 units SuperTaq (Taq-polymerase) in Superbuffer (SphaeroQ). AFLP reactions were performed according to the touchdown PCR profile. For gel electrophoresis 0.5 to 0.7 µl from each sample was loaded on a 5.5% denaturing polyacrylamide gel (5.5% Ready to use Gel Matrix, KB Plus, Westburg).

The majority of the markers in the AFLP analyses were scored dominantly, except for the analyses of the BC_4 generation, where all markers were scored codominantly by Keygene, the Netherlands, using Quantar software (developed at Keygene).

Genotype presentation

During Marker Assisted Selection, all plants were genotyped with on average 220 markers by using six to eight AFLP primer combinations. When a plant with a single introgression was obtained, an extensive AFLP marker analysis was performed with minimal three extra primer combinations to once again verify the absence of other introgressions. For the Graphical Genotype Analysis we used the genetic linkage map of *L. saligna* × *L. sativa* with 476 AFLP markers, an average spacing between markers of 1.8 cM and a maximal distance of 16 cM (Jeuken et al. 2001).

RESULTS

Backcross program

From a single F_1 plant, the BC_1 generation was obtained by backcrossing with *L. sativa* Olof. Backcrosses on eleven random BC_1 plants yielded the BC_2 generation. Backcrosses on 13 BC_2 plants, descending from all 11 BC_1 parents, yielded the BC_3 generation. Seventeen random BC_3 plants, descending from all 13 BC_2 parents were backcrossed to obtain the BC_4. All backcrosses from F_1 to BC_4 were made randomly without selection on genotype or phenotype, because a major prerequisite, a linkage map of *L. saligna* × *L. sativa*, was not available yet. At the time that the BC_4 generation was obtained a linkage map had been constructed and therefore, genotype analysis and selection became possible.

From the BC_4 generation 84 individuals were analyzed with 267 codominant AFLP markers. The percentage of recurrent parent genome in the BC_4 plants ranged from

77% to 100% (Figure 2). The average recurrent parent genome per BC_4 plant was 95%, which did not deviate much from the theoretically expected 97% (31/32).
Based on the graphical genotypes, 63% to 69% of the *L. saligna* genome was present in the BC_4 (Figure 3). The six- percent inaccuracy was due to the distances between two markers flanking a recombination event (between the outermost analyzed marker

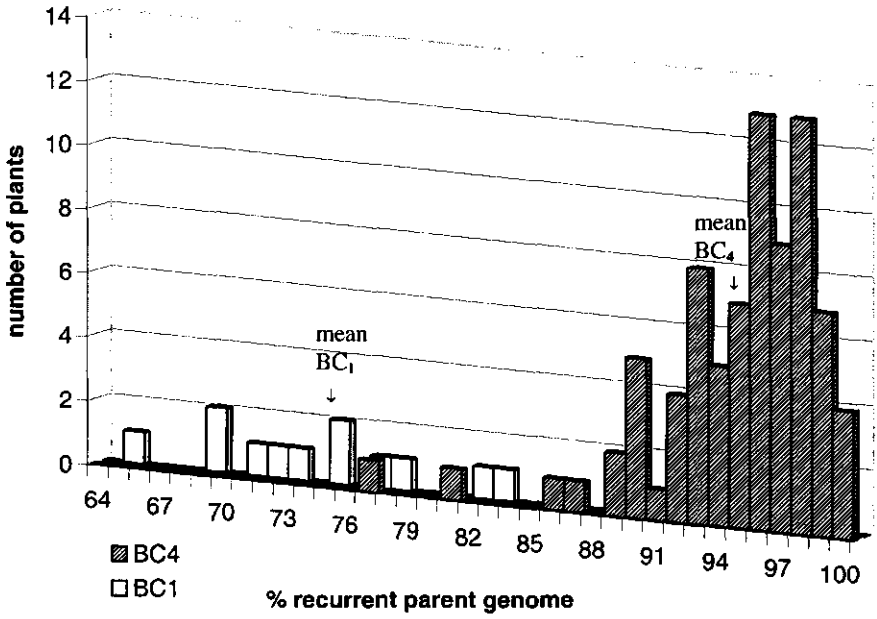
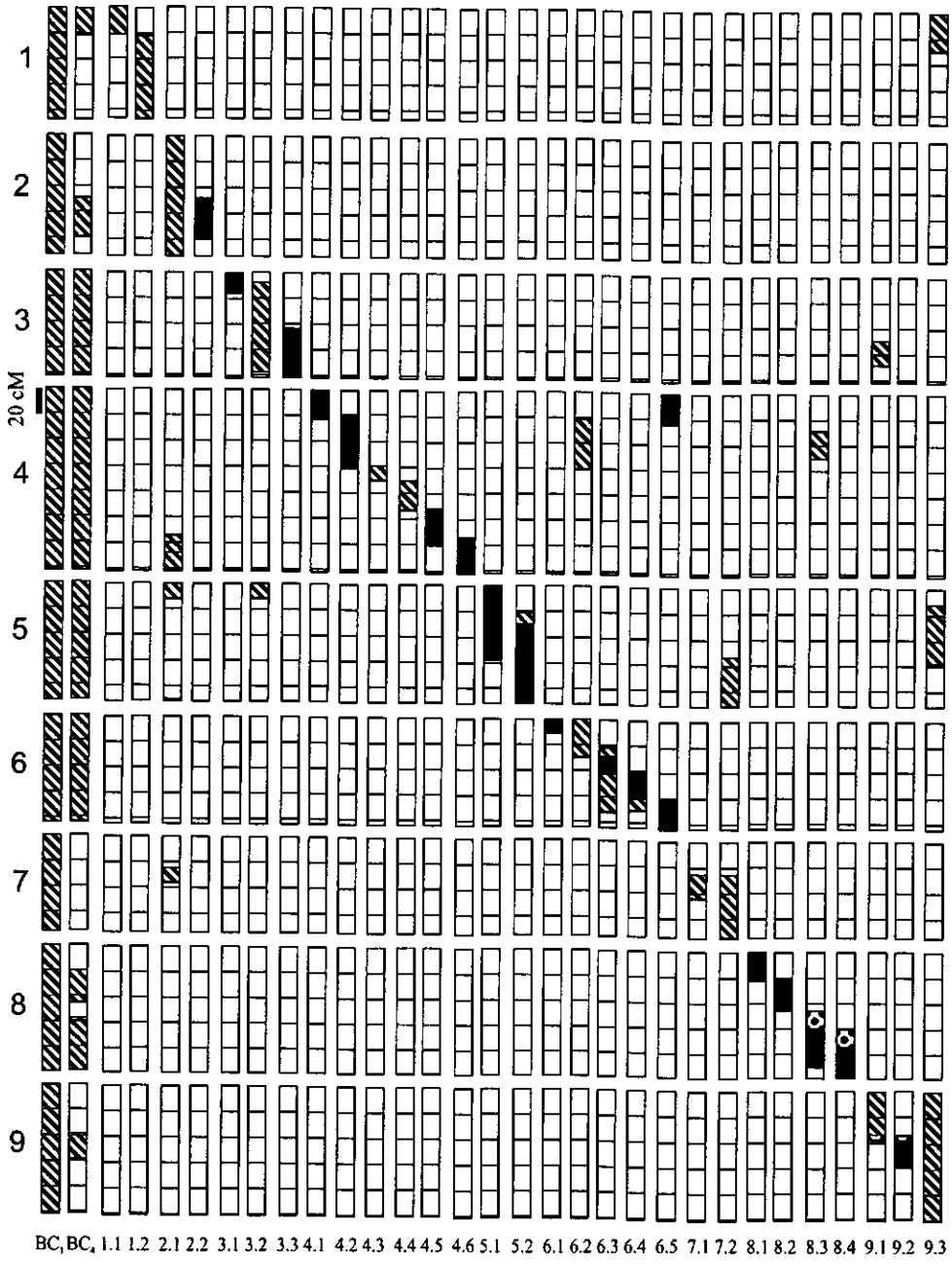


FIGURE 2. Distribution of percentage of recurrent parent genome values in the BC_1 and BC_4 generation. *L. sativa* Olof is the recurrent parent and *L. saligna* CGN 5271 is the wild parent.

FIGURE 3. (NEXT PAGE) Presentation of the genome coverage of the BC_1 and the BC_4 generation and presentation of the genotypes of 29 backcross lines (line 1.1 to 9.3) that cover more than 95 % of the *L. saligna* genome. Vertically the nine chromosomes of lettuce are drawn. Within the chromosomes, lines mark distances of 20 cM. The BC_1 and BC_4 genome coverage and the 29 backcross lines are presented horizontally. The shadowed area indicates introgressions. Genotypes are represented by color. White represents homozygous *L. sativa* Olof, black is homozygous *L. saligna*, grey is heterozygous and the dotted design indicates unknown genotype, as not enough markers are analyzed in that region. Introgressions of *L. saligna* were presented till the outermost analyzed marker. Therefore, this is a minimal representation of the genome coverage, as the genotype between the outermost marker of the introgression and the first adjacent marker outside the introgression, was not identified.


1
2
3
20 cM
4
5
6
7
8
9
BC1BC4 1.1 1.2 2.1 2.2 3.1 3.2 3.3 4.1 4.2 4.3 4.4 4.5 4.6 5.1 5.2 6.1 6.2 6.3 6.4 6.5 7.1 7.2 8.1 8.2 8.3 8.4 9.1 9.2 9.3

in the introgression and the first adjacent analyzed marker outside the introgression). So, 30-36% of the *L. saligna* genome was lacking and had to be retrieved from earlier generations (Figure 3).

Therefore, AFLP analyses were performed on 49 BC_3 plants. The only introgression that was lacking in the BC_4 and could be retrieved from a BC_3S_1 plant was Chromosome 8 from 45-84 cM. The other introgressions that were lacking in the BC_4 were also absent in the BC_3 generation (data not shown).

Further, in the BC_1 generation all 12 individuals were used for AFLP analysis with 125 dominant markers. The average recurrent parent genome per BC_1 plant was 74 (± 9) %, which did not deviate much from the theoretically expected 75% (Figure 2). Graphical genotype analyses on the BC_1 showed that the complete genome of *L. saligna* was covered in the 11 BC_1 plants that had been backcrossed to BC_2 (Figure 3). Chromosome regions of *L. saligna* were presented in at least one BC_1 plant and in at most ten BC_1 plants. The following regions were most underrepresented in the BC_1: Chromosome 2 from 0 to 23 cM and Chromosome 7 from 0 to 25 cM were present in one plant only; Chromosome 9 was never found to be involved in intrachromosomal recombination. This chromosome was either present as completely derived from the *L. saligna* parent (in two progeny plants) or completely present as *L. sativa* derived (ten progeny plants).

Six introgressions that were lacking in the BC_4 generation could be retrieved from BC_1S_1 plants. The region on Chromosome 7 from 0 to 25 cM was not retrieved. This introgression was present in one BC_1 plant, but was absent in all eight genotyped BC_1S_1 plants. So, as the BC_1S_1 plants were lost, this chromosome fragment could not be retrieved anymore.

The selected BC_3S_1 and BC_1S_1 plants were backcrossed and selected for by MAS to develop BILs containing these introgressions.

29 lines with minimal introgressions and maximal genome coverage

At this stage in the backcross program a selection was made for the minimal number of lines with a minimal number of introgressions and with a maximal coverage of homozygous *L. saligna* genome. This resulted in 29 lines that covered at least 95% of the genome of *L. saligna* (Figure 3). Sixteen BILs (2.2, 3.1, 3.3, 4.1, 4.2, 4.5, 4.6, 5.1, 5.2, 6.1, 6.3, 6.4, 8.1, 8.2, 8.4 and 9.2) covered in total 51% of the *L. saligna* genome. On average, each BIL contained 3.4% (=29 cM) of the *L. saligna* genome. Five lines (1.1, 1.2, 4.3, 4.4 and 7.1) had a single heterozygous introgression, covering in total 16% of the genome. Seven lines (2.1, 3.2, 6.2, 7.2, 8.3, 9.1 and 9.3) had two or more introgressions, from which the unique chromosome regions cover at least 28% of the *L. saligna* genome that was not yet covered by lines with a single introgression.

Distorted segregation ratios

During development of the BILs, the progeny of several backcross lines provided information about segregation ratios, which could be compared to former observed skewed segregations in the F_2 population (Jeuken et al. 2001). To determine skewness

for a specific genome region, only sets of backcrossed plants consisting of at least 21 informative plants were taken into account (Table 1). In this table the observed and expected segregation ratio's of three *L. saligna* introgressions are shown for the F_2 population and for sets of backcross populations obtained mainly by backcrosses with *L. sativa* Olof as a father and once by reciprocal backcrosses.

In the F_2 population several regions showed distorted segregation ratio's deviant from the expected Mendelian1: 2:1 ratio (Jeuken et al 2001). These skewnesses have been quantified in F_2 segregation ratio's by DNA markers on these regions.

The introgression on Chromosome 5 from 0 to 15 cM showed in the F_2 population a distorted segregation ratio of 3: 37: 49 (homozygous *L. sativa*: heterozygous: homozygous *L. saligna*). For the marker with the fewest missing data in this region allele frequencies were calculated from the F_2 segregation ratio: *L. sativa* allele=0.24 and *L. saligna* allele = 0.76. It seems that a gene closely linked to the *L. sativa* allele of this marker is responsible for a lower pollen, egg cell or zygote fitness than the *L. saligna* gene. Such lower fitness should be expected to distort segregations also in backcross lines in a similar way. Instead of a 1:1 ratio, we expect a 24: 76 ratio in alleles (*L. sativa*: *L. saligna*), resulting in a 6:17 ratio in genotypes (homozygous *L. sativa*: heterozygous), if the backcross population contains 23 plants.

TABLE 1. Comparison of segregation ratios between/among backcross lines and the F_2 population. The backcross lines were genotyped with 195 AFLP markers.
Significance level threshold p= 0.05. Genotype indications: a= homozygous *L. sativa*, h= heterozygous, b= homozygous *L. saligna*.

Position of introgression, Chromosome and region in cM, and most informative AFLP marker	F_2 population		Backcross lines			
	Observed segregation ratio a:h:b, significantly different from Mendelian 1:2:1 ratio	Allele frequency M= *L. sativa* m= *L. saligna* based on the most informative marker	Expected segregation ratio a:h, based on allele frequencies in F_2, instead of a Mendelian 1:1 ratio	Observed segregation ratio a:h, (direction of cross leading to the backcross line)	χ^2 (df 1) if expected= 1:1 ratio	χ^2 (df 1) if expected ratio= based on allele frequencies in F_2
C5, 0-13 E35M49-106satB	3:37:49 xxxxx	M= 0.24 m= 0.76	6:17 (n=23) 5:16 (n=21)	8:15, (Olof ♂) 1:20, (Olof ♀)	2.1, ns 17.2, xxxx	1.3, ns 4.2, x
C7, 25-73 E45M48-259sal	37:45:8 xxx	M= 0.66 m= 0.34	22:12 (n= 44)	23:11, (Olof ♂)	4.2, x	0.1, ns
C9, 0-33 E54M48-264sal	71:47:0 xxxxxx	M= 0.80 m=0.20	30:8 (n= 38)	19:19, (Olof ♂)	0, ns	21.4 xxxxx

Legend: each chromosome interval was represented by the most informative AFLP marker

ns	not significant
x	p=0.05
xx	p=0.005
xxx	p=0.0005
xxxx	p=0.00005
xxxxx	p<0.000005

We observed a segregation ratio of 8:15 for the introgression on Chromosome 5 in two backcross lines ($BC_1S_1BC_2$ and BC_4) obtained by backcrossing with *L. sativa* Olof as a pollen donor. This observed segregation ratio was not significantly different from the expected ratio 6:17 (based on allele frequencies in the F_2 population) nor from the Mendelian segregation of 1:1. However, in three BC_4 lines, obtained from a backcross with the three BC_3 plants as a pollen donor (and *L. sativa* Olof as mother!), we observed a skewed segregation. This was significantly different from the 1:1 Mendelian ratio and also from the expected skewed ratio based on the F_2 population. Pollen with *L. saligna* alleles on Chromosome 5 from 0 to 13 cM had a 20 times higher fitness compared to pollen with only *L. sativa* alleles in this region (Table 1). A higher fitness for egg cells with *L. saligna* alleles in this region was not observed when the BC parent was used as a female parent.

The introgression region on Chromosome 7 from 25-73 cM, present in two backcross lines ($BC_1S_1BC_1$ and $BC_1S_1BC_2$), showed skewness while *L. sativa* Olof was used as the pollen donor, that was similar to the skewness in the F_2 population. This can be explained by assuming that egg cells containing *L. saligna* alleles at Chromosome 7 from 25-73 cM had a lower fitness. Results from reciprocal backcrosses were not available.

The region in Chromosome 9 from 0 to 33 cM showed severe skewness in the F_2 population, as no F_2 plants were homozygous *L. saligna* at this region. This skewness resulted in allele frequencies of 0.80 for the *L. sativa* allele and 0.20 for the *L. saligna* allele. However, two backcross lines ($BC_1S_1BC_1$ and $BC_1S_1BC_2$) did not show skewed segregation for this introgression when *L. sativa* Olof was used as the pollen donor. This suggests that the distorted segregation in the F_2 was due to reduced fitness of pollen with *L. saligna* alleles for the introgression, while the fitness of the egg cell was unaffected by these *L. saligna* alleles.

Finally, a remarkable distorted segregation was observed in Line 1.1 with a heterozygous introgression of Chromosome 1 from 0-21 cM (Figure 3). Progeny of this line segregated 10:25:0 (homozygous *L. sativa*: heterozygous: homozygous *L. saligna*). It is most striking that not a single homozygous plant was detected and a BIL homozygous for this chromosome region cannot be obtained, while in the F_2 population a normal segregation was observed.

Morphological traits

During the development of the BILs some morphological characteristics from *L. saligna* were clearly segregating within and between backcross lines. Some of these traits were easily mapped since they were invariably associated with *L. saligna* alleles of particular AFLP markers. Accordingly, they were used as morphological markers (Table 2). For example, in *L. sativa* Olof the leaf apex is round as in most *L. sativa* cultivars. In *L. saligna* the leaf apex is pointed. This characteristic, pointed leaf apex, is dominant and was mapped on Chromosome 5 in a region from 0 to 13 cM. This trait is expressed in BIL 5.1 and in the Lines 2.1 and 3.2 (Figure 3).

TABLE 2. Overview of atypical phenotypes of BILs and lines deviant from *L. sativa* Olof.

BIL/ line	Chromosome fragment (cM)	Phenotype
3.1	C3, 0-16	reflexed involucre (mapped at 0 to 3 cM)
4.2	C4, 18-61	long, dark green leaves, some leaves are twisted upside down
4.6	C4, 114-142	blistered leaves
5.1	C5, 0-57	pointed leaf apex (mapped at 0 to 15 cM)
5.2	C5, 22-90	highly branching
7.2	C7, 45-72	early bolting (mapped at Chromosome 7, 45 to 60 cM)
8.4	C8, 76-99	brown seeds instead of black like *L. saligna* CGN 5271 or white like *L. sativa* Olof
9.1/9.3	C9,0-33	necroses on leaves and stem (mapped at 0 to 4 cM)
9.2	C9, 38-58	irregular and not-waxy leaf surface; irregular leaf color distribution with many light green to white areas.

L. sativa has a nonreflexed involucre causing retention of mature seeds. *L. saligna* has a reflexed involucre at maturity, promoting seed dispersal. This trait was mapped on Chromosome 3 from 0 to 3 cM and is expressed in Line 3.1.

Some other morphological characteristics were noticed in backcross lines but not in *L. saligna* nor in *L. sativa* Olof. For example, on Chromosome 9 from 0 to 4 cM an atypical characteristic is spontaneous necroses on leaves and stem in adult plants. This phenotype is expressed in Line 9.1 and 9.3. Another trait was early bolting, associated with an introgression of Chromosome 7 from 45 to 60 cM.

Some of the 16 homozygous BILs showed quantitative variation between BILs for some traits, like number of branches, leaf shape, leaf color, leaf surface and seed color (Table 2). The seed set of most BILs was similar to *L. sativa* Olof, with two exceptions: BIL 4.2 had a low seed set and in contrast, BIL 4.6 was very fertile as it showed an almost double number of seeds per capitulum (20 seeds per capitulum on average) compared to *L. sativa* Olof (11 seeds).

Extra DNA marker information

Some AFLP markers in the F_2 population could not be scored due to closely migrating or faint amplification products. As BILs are genetically very similar, polymorphic amplification products can be scored more unambiguously. By using BILs, we identified 117 additional clearly segregating AFLP markers, which were not identified in the F_2 population while the same AFLP primer combinations were used (data not shown). So, these BILs are very useful for accurately assigning markers to chromosome fragments.

DISCUSSION

In the present study we have developed a set of 29 lines covering at least 95 percent of the *L. saligna* genome, despite unfavorable phenomena of the F_2 population like sterility, lower vigour and distorted segregations. This was achieved in only four to five backcross generations and one selfing generation, despite the fact that a genetic linkage map only became available after three backcross generations. Our approach was more efficient than some others that mostly included fewer backcross generations and more selfing generations like $BC_1S_6BC_3S_1$ (Eshed and Zamir 1994), BC_2S_3 (Monforte and Tanksley 2000), BC_2S_6 (Chetelat and Meglic 2000) and BC_1S_5 (Lin et al. 1998).

The genetic linkage map that we used for MAS was accurate since the chromosome regions of the BILs confirmed the marker orders in the F_2 linkage map. We cannot completely exclude the possibility of extremely small unnoticed introgressions of less than 4 cM (854 cM/220 markers).

L. saligna genome coverage in BC_1 and BC_4

The average recurrent parent genome coverage in the BC_1 and BC_4 was very similar to the expected values of 75% and 97%, respectively. This is an indication that there is no general preference for alleles of one of the parents.

During backcrossing without Marker Assisted Selection from BC_1 to BC_4 30-36 % of the *L. saligna* genome was lost. Most of the lost introgressions were also not present in the BC_3. This could be due to the small sampling sizes in the BC_1, BC_2 and BC_3 generations. However, the backcrosses from BC_2 to BC_3 to BC_4 have been made only unidirectionally with *L. sativa* Olof as a mother. This unidirectional backcrossing could have selected against genes affecting reproductive processes such as egg cell, pollen, or zygote fitness. From the eight missed introgressions in the BC_4, four showed a distorted segregation with an excess of *L. sativa* alleles in the F_2 population (Jeuken et al. 2001). These introgressions were Chromosome 9 from 0-33 cM and Chromosome 9 from 58-101 cM and Chromosome 7, 0-25 and Chromosome 7, 25-75 cM. This suggests that selection had taken place via genes effecting pollen fitness or zygote viability. The lack of the other introgressions, with no distorted segregation ratio in the F_2 population, could possibly be explained by the small sample sizes. To prevent losing introgressions due to differences in pollen fitness it is recommended to use the recurrent parent as a male parent. Another way to prevent losing introgressions would be using Marker Assisted Selection during the backcross program in each generation from BC_1 till BC_4. In that way a missing introgression would have been noticed immediately and it would have taken little effort to retrieve it from an earlier generation. So, the best solution is to use Marker Assisted Selection and use the recurrent parent as a father.

Completion of a set of BILs

At least 95% of the *L. saligna* genome was already covered in these 29 lines. We expect to reach maximally 97% coverage. The only region that is impossible to introgress is the top of Chromosome 7 from 0 to 25 cM. The progeny (n=8) of the only BC_1 plant harboring this introgression did not contain the segment.

Since from the 29 selected lines, 21 lines have a single introgression and 16 are BILs, the perspectives to complete the set of BILs are great. However, it might be possible that regions that showed extreme distorted segregation with a preference for *L. sativa* alleles cannot be obtained homozygously, like the top of Chromosome 9. This also holds for Line 1.1, carrying the top of Chromosome 1, that could not produce a homozygous plant in a progeny of 35 plants obtained from selfing a heterozygous plant. This absence of some chromosome fragments in a final set of BILs was also observed in the development of other sets of BILs and is ascribed to lethal combinations of genes from the two donor parents (Chetelat and Meglic 2000, Monforte and Tanksley 2000).

The 16 BILs with one homozygous introgression contained on average 3.4% (29 cM) of the *L. saligna* genome, which was between the expected 6.3 % (1/16) and 3.1% (1/32), based on four to five backcrosses and one selfing. Again, there is no indication of an overall preference for alleles of one of the parents. To completely cover the remaining 46% (97-51=46%) of the *L. saligna* genome 14 BILs are required with a similar average introgression size of 29 cM.

Distorted segregation ratio's

The uniqueness of the study on the segregation ratios of three chromosome regions was that these could be compared between a selfing progeny (F_2) and a backcross progeny and in one case also with the reciprocal backcross progeny. The distorted segregations in the three studied introgressions on Chromosome 5, 7 and 9 are caused by genes or alleles from *L. saligna* that effected pollen or egg cell fitness.

Based on the distribution of recurrent parent genome in the BC_1 and BC_4 (Figure 2) and the study on the segregation ratios (Table 1), there is no evidence for a general preference for alleles of one of the parents. This is in contrast with some studies in BIL development (Monforte and Tanksley 2000, Chetelat and Meglic 2000), in which a deficit of the wild allele has been observed.

Morphological characteristics

Pointed leaf apex was identified in backcross lines, in progeny from selfed lines and in BIL 5.1. As this trait was associated with homozygous and heterozygous introgressions, it was dominant. Probably, it was monogenic or otherwise explained by closely linked genes within 13 cM map distance. This is in agreement with asparagus lettuce, in which a single dominant gene has been found expressing pointed leaf apex (Lindqvist 1960). This morphological trait can be used as an easy scorable marker as it was easily recognizable in an early stage of plant development.

Reflexed involucre was also identified in lines with the specific introgression in heterozygous or homozygous state. Therefore, this trait was also dominant and probably monogenic. Lindqvist also found that the *L. saligna* allele for this trait was dominant over the *L. sativa* allele in F_1 plants (Lindqvist 1956). Reflexed involucre is easy to score, but in a very late plant stage when seed is maturing. This makes it less valuable as a morphological marker. The atypical phenotypes of some other BILs can be used as morphological marker too.

Perspectives of BILs

This study demonstrates that BILs can be used for mapping simple morphological traits and for identifying and mapping region-specific molecular markers. Other perspectives with high potentials for the lettuce BILs are mapping Expressed Sequence Tags (ESTs) of *L. sativa* and *L. saligna* and the genetic dissection of agriculturally valuable traits including quantitative traits with complex inheritance patterns like the *Bremia* resistance of *L. saligna*. These quantitative traits are usually difficult to study in other population types.

ACKNOWLEDGEMENT

We thank P. Stam and R.E. Niks for critically reading the manuscript and their advisory support in this study. We are thankful for the financial and advisory support of the Dutch lettuce breeding companies. We thank Keygene for co-operation in AFLP scoring.

5

BILs IN LETTUCE REVEAL QTL FOR DOWNY MILDEW RESISTANCE THAT REMAINED HIDDEN IN AN F_2 MAPPING APPROACH

Marieke Jeuken and Pim Lindhout

Our goal was to genetically characterize the non-host resistance of *L. saligna* (wild lettuce) to lettuce downy mildew (*Bremia lactucae*). The results of an F_2 QTL mapping method were verified by a BIL mapping method. Backcross Inbred Lines with single homozygous introgressions of *L. saligna* in a *L. sativa* background were tested for resistance to *Bremia* race NL16. The nine lines (BILs) tested covered together 31% of the *L. saligna* parental genome. Two resistance loci detected in the F_2 population (*R39* and *RBQ3*) were confirmed in the disease test on the BILs and the phenotypic effects of the resistance genes were estimated to be larger in the BILs. No conclusive comparisons of *RBQ2* could be made, as the introgression in the backcross line was not in homozygous state. *RBQ1* was not tested. *R39* was a dominant gene, which gave a complete resistance against *Bremia* race NL16. *RBQ3* reduced the infection severity of the susceptible *L. sativa* by 49%, (43% percent points infection severity) ten days post inoculation. The BIL method revealed a new resistance locus on Chromosome 8 with a 77% reduction on the infection severity compared to the susceptible control (68% percent points infection severity reduction) ten days post inoculation. We conclude that the BIL mapping method can reveal new QTLs unnoticed in the F_2 mapping method and allows a quantification of the gene effect in a *L. sativa* background.

L. sativa (lettuce) is host for lettuce downy mildew (*Bremia lactucae*; Crute 1992). Resistance to this pathogen is one of the most important breeding goals for all lettuce types (Reinink 1999). As monogenic resistance genes to *Bremia* (*Dm* genes) are rendered ineffective rapidly, other types of resistance are searched for as a more durable alternative. From the *Lactuca* species that can be crossed with cultivated

lettuce only *L. saligna* is completely resistant to downy mildew and might be considered a non-host (Norwood 1981, Gustafsson 1989, Lebeda and Boukema 1991, Bonnier et al.1992). The non-host resistance of *L. saligna* was for the first time genetically dissected in an F_2 population from a cross of *L. saligna* with a susceptible *L. sativa* (Jeuken and Lindhout 2002). This revealed one *Dm*-like gene (*R39*) that attributed nearly complete race-specific resistance to *Bremia* race NL16 but not to race NL14 and three QTLs (*RBQ1*, *RBQ2* and *RBQ3*) that were effective to both races (and may be race non-specific; Jeuken and Lindhout 2002). However, small population size, extreme morphological differences within the segregating population, extreme distorted segregation ratios at certain chromosome regions and strong epistatic interactions of genes can limit the accuracy of an F_2 QTL mapping strategy. Therefore another strategy was used to dissect and map resistance genes from *L. saligna*, the Backcross Inbred Line (BIL) population method. A BIL is a line with a single homozygous introgression of a donor accession or donor species in a recipient species background. The length of an introgression is about a quarter to half a chromosome. An ideal set of BILs covers the complete genome of the donor species. The expected major advantages of BIL populations are: 1) The high genetical and morphological similarity between lines allows more accurate estimates of the quantitative trait. 2) The homozygous nature of BILs allows unlimited repeats of measurements, also in different seasons and environments (QTL × environment interaction) and in different genetic backgrounds (QTL × genetic background interaction). Some of these aspects were described in a study on yield associated QTLs in a tomato (*L. esculentum*) BIL with an introgression of *L. pennellii* (Eshed and Zamir 1995).

We developed a population of backcross inbred lines with single homozygous introgressions of *L. saligna* in a *L. sativa* background (Chapter 4). In this preliminary study a selection of BILs with one introgression and one backcross line with four introgressions were tested for resistance to *Bremia* race NL16. Our goals were 1) To confirm *R39* and two QTLs for resistance (*RBQ2* and *RBQ3*) found in the F_2 population; 2) To quantify the resistance effect (based on infection severity score) of *R39*, *RBQ2* and *RBQ3* in lines with a nearly pure *L. sativa* background; 3) To identify new resistance genes from *L. saligna*, which were not detected in the F_2 population; 4) To evaluate the BIL mapping method in lettuce.

A third QTL for resistance to *Bremia*, *RBQ1*, was not tested as no BIL was available covering Chromosome 7 at the *RBQ1* locus.

MATERIAL AND METHODS

Lines

From a population of backcross inbred lines, seeds of five lines with one homozygous introgression and two lines with one heterozygous introgression were available for a *Bremia* disease test. Lines with a homozygous introgression from *L. saligna* are referred to as BILs, while other lines are referred to as Lines. Seed availability at that moment was the only criterion for selection of BILs for this disease test. Five BILs (3, 4, 5, 6 and 8; Figure 1) with

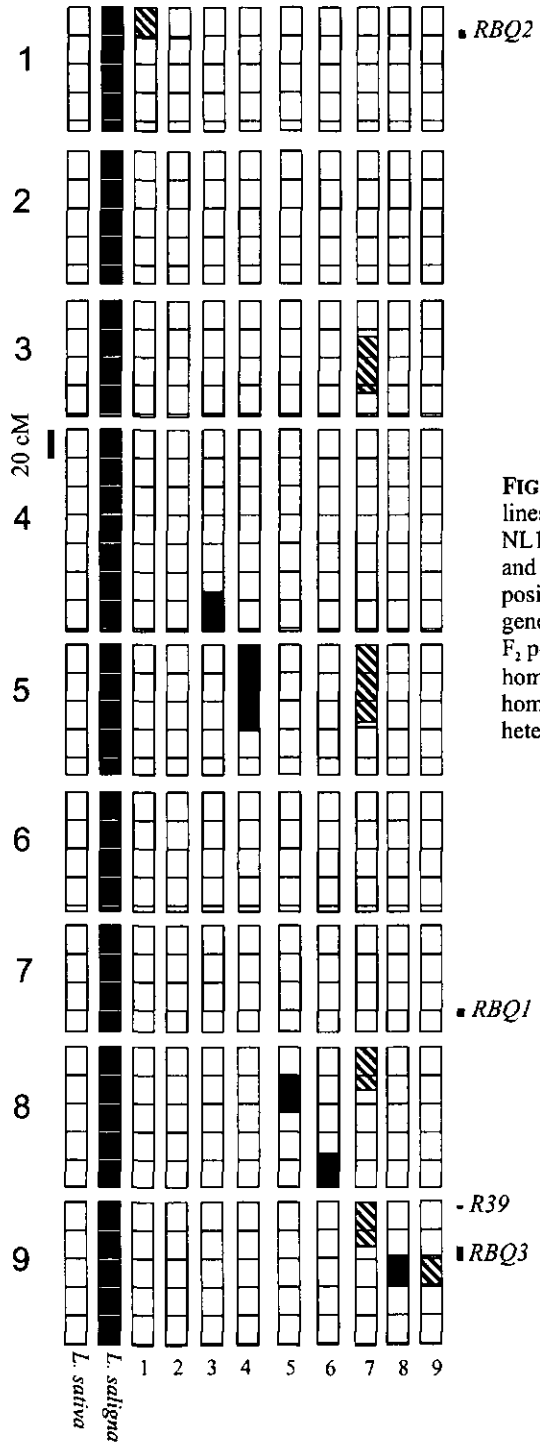


FIGURE 1. Genomic presentation of lettuce lines used in *Bremia* disease test with race NL16. Chromosomes are shown vertically and lines are presented horizontally. The position and mapping interval of resistance genes *R39*, *RBQ2* and *RBQ3* based on the F_2 population are indicated. White represents homozygous *L. sativa* Olof, black is homozygous *L. saligna* and grey is heterozygous.

observed to be conferred by *RBQ3*. However, fourteen days after inoculation, plants with *RBQ3* did not show a significant difference in infection severity with the susceptible control. We conclude that *RBQ3* slowed down the infection process of *Bremia*. The delay of the infection process was visible 10 days post inoculation but not anymore 14 days post inoculation. The observed decrease in infection severity ten days post inoculation was higher than was measured in the F_2 population for *RBQ3*. (Table 2)

The other class of plants of Line 9 consisted of nine plants having a similar infection severity score as the susceptible parent, suggesting no resistance allele. Furthermore, no resistance effect was observed when the susceptible control was compared with the infection severity scores of all 13 plants of Line 9, which should represent the average resistance effect of one *RBQ3* allele (when a non-distorted segregation ratio was assumed). Therefore, it seemed that there was no additive effect for *RBQ3* alleles and the inheritance would be recessive. However, distorted segregation ratios or skewness by change in Line 9 could not be excluded because the line was not analyzed with DNA markers. Therefore, to measure very precisely the effect of one allele of *RBQ3*, a disease test experiment is preferred with many plants that are confirmed to be heterozygous for *RBQ3* by DNA markers.

3) Identification of new resistance genes

BIL 5 with an introgression of Chromosome 8, not known to harbor loci for *Bremia* resistance, clearly showed a substantially reduced infection severity score, ten and fourteen days after inoculation, indicating resistance to *Bremia* race NL16. The gene(s) from *L. saligna* responsible for this resistance should be located on the introgression segment on Chromosome 8 between 21 and 45 cM. No QTL for *Bremia* resistance was mapped on Chromosome 8 by using the F_2 population. The resistance found in BIL 5 is likely to represent an additional QTL, designated *RBQ4*.

TABLE 2. Effects of *Bremia* resistance genes on infection severity scores were quantified in BILs and in an F_2 population ten days post inoculation. The reduction in percent points on the average *Bremia* infection severity in BILs was shown compared to the susceptible *L. sativa* Olof (infection severity of 88%). The difference between the average *Bremia* infection severity of two classes of F_2 plants with and without the gene(s)/allele(s) was shown in Units (according Jeuken and Lindhout 2002) and in percentages (Unit ×25=..%).

gene	# alleles	BILs	F_2 (Units)	F_2
R39	1	88%	2.1	53%
R39	2	N.D.	N.D.	N.D.
RBQ2	1	0%	0.5	13%
RBQ2	2	N.D.	1.0	25%
RBQ3	1	0%	0.5	13%
RBQ3	2	43%[a]	1.0	25%

a) This average reduction on infection severity is based on BIL 8 and the resistant class of Line 9 (88-45=43%)

Remarkably, this introgression of Chromosome 8, which was heterozygously present in F_2 plant 35 and which was segregating in the corresponding F_3 line 35, segregated for resistance (3 intermediate resistant plants out of 7) while the F_2 plant 35 was highly susceptible to two *Bremia* races (NL14 and NL16, Jeuken and Lindhout 2002). Therefore this gene *RBQ4* might be recessive.

In addition, Line 3 with an introgression on Chromosome 4, also not known to harbor loci for *Bremia* resistance, showed reduced infection severity scores, especially ten days post inoculation. However the surface of the leaves of these plants and also their leaf discs were blistered. The leaf discs did not touch the filter paper completely and were probably less exposed to the humidity from the filter paper. Therefore, the leaf disc conditions were different compared to the leaf discs of other lines and could be the cause of lower infection severity scores. Disease tests on whole plants of this BIL have to be performed to make a definite conclusion about occurrence of resistance in this genotype to *Bremia*.

4) Evaluation of the BIL mapping method

The resistance conferred by *R39* and *RBQ3* was confirmed in the BILs. One allele of *R39* from *L. saligna* caused complete resistance to *Bremia* NL16, while in the F_2 population it caused a nearly complete resistance (Table 2). Two alleles of *RBQ3* had a larger effect in reduction of infection severity measured in a BIL than in an F_2 population (Table 2). We assume that the conclusion about the resistance effect of *R39* and *RBQ3* based on the BIL disease tests is more reliable and holds more true than the conclusion about the resistance effect of *R39* and *RBQ3* based on the F_2 population. The reason for this is that in the disease tests on an F_2 population not only QTLs for resistance segregate but also segregation of the genetic background occurs, which may enhance or reduce the expression of these QTLs. Furthermore, extreme morphological differences of F_2 plants may influence the disease tests. In contrast, disease tests on BILs were done on plants with a more uniform genetical and morphological background. Therefore, we assume that the quantification of the effect of a single resistance gene is more precise in a line with a more pure *L. sativa* background like a BIL than in a F_2 genotype. This is also more relevant for lettuce breeders.

The resistance of *RBQ2* has not been confirmed in the segregating Line 1. This could be due to the distorted segregation of this line in not producing a plant with a homozygous introgression or because the effect of one allele of *RBQ2* was too small. The definite quantification of the effect of *RBQ2* awaits marker analyses of individuals to identify genotypes with and without of *RBQ2* or eventually a BIL with *RBQ2* in a homozygous state.

The discovery of a new resistance gene *RBQ4* on Chromosome 8 showed that BILs are very useful for detection of new resistance genes. It also showed that resistance genes with a substantial effect could go unnoticed in F_2 populations. The most likely explanation for missing *RBQ4* in the F_2 population is that for Chromosome 8 a distorted segregation ratio of 22:61:3 (homozygous *L. sativa*: heterozygous: homozygous *L. saligna*; 40 individuals not genotyped) was found, indicating an

excess of heterozygotes and an underrepresentation of genotypes with two *L. saligna* alleles. This also suggests no additive effect for *RBQ4* alleles and a recessive inheritance.

CONCLUSION

R39 is a monogenic dominant resistance gene conferring a complete resistance against *Bremia* NL16.
RBQ3 is a resistance gene, which causes sparser sporulation of *Bremia* and a slower infection process of *Bremia*. This results in a 49% reduction of infection severity ten days post inoculation, but no reduction of infection severity 14 days post inoculation.
A new resistance gene(s) *RBQ4*, probably recessive, from *L. saligna* was identified in BIL 5 at Chromosome 8, 21 to 45 cM with a reduced infection severity of 77% and 59%, ten and fourteen days post inoculation.
From the present results it may be concluded that BILs could detect new genes that were unnoticed in F_2 populations.

ACKNOWLEDGEMENT

We thank P. Stam and R.E. Niks for critically reading the manuscript and their advisory support in this study.

6

ARE THE SAME QUANTITATIVE RESISTANCE GENES AGAINST DOWNY MILDEW PRESENT IN TWO ACCESSIONS OF *L. SALIGNA*?

Marieke Jeuken and Pim Lindhout

L. saligna **is a non-host to lettuce downy mildew. Previously we showed that *L. saligna* CGN 5271 harbors one *Dm* gene and four QTLs for resistance. In the present study we analyzed the non-host resistance of *L. saligna* CGN 11341 by disease tests and DNA marker analyses on an F_2 and BC_1 population. Disease tests with two *Bremia* races showed a wide range of infection severity scores from resistant to susceptible to both races. The majority of plants had a similar resistance level to both *Bremia* races. These findings imply that the resistance of *L. saligna* is quantitatively expressed and is probably race non-specific. A few F_2 and BC_1 plants were completely resistant against *Bremia* race NL16 and rather susceptible to race NL14. This race-specific resistance was explained by a major resistance gene that was located on Chromosome 9. This gene was designated *R39b*, as it may be different from *R39*, detected in *L. saligna* CGN 5271.**

No additional QTLs were detected in the F_2 population, which is probably due to the small population size. However, F_2 plants with *L. saligna* CGN 11341 alleles at loci of four putative QTLs for resistance mapped in *L. saligna* CGN 5271 were more resistant than F_2 plants without *L. saligna* alleles for these putative QTLs. In conclusion, we state that it is very likely that the same genes explain the resistances to *Bremia* in both *L. saligna's*. A backcross program for a set of Backcross Inbred Lines (BIL) that cover *R39b* and loci for putative QTLs, is in progress.

Several *Lactuca* species are host for lettuce downy mildew (*Bremia lactucae)* (Lebeda and Syrovatko 1988). Cultivated lettuce (*L. sativa*) and two related species (*L. serriola* and *L. virosa*) show a large variation in resistance to downy mildew. These resistances are mainly explained by *Dm* genes that show gene-for-gene relationships

with races of *Bremia* (Bonnier et al.1992). Of the non-hosts to lettuce downy mildew, *Lactuca saligna* is the best-studied *Lactuca* species. *L. saligna* is completely resistant to *Bremia* and can be crossed with cultivated lettuce (Norwood 1981, Gustafsson 1989, Lebeda and Boukema 1991, Bonnier et al.1992). It may be one of the very few examples of a species with a non-host status that can be crossed with a host species, and hence may be used to investigate the genetic basis of non-host resistance. It is not clear whether "non-host" resistance is explained by resistance genes of the non-host that recognize avirulence genes of inappropriate pathogens or by nonspecific general defenses induced by nonspecific stimuli of the pathogen (Heath 2001). Non-host resistance may also be based on a combination of these types of resistances. For more discussion on non-host resistance we refer to the General Introduction (Chapter 1). In order to genetically dissect the non-host resistance of *L. saligna* to *Bremia*, a QTL mapping study has previously been performed with two *Bremia* races tested on an F_2 population, obtained from a cross between *L. saligna* CGN 5271 and a susceptible *L. sativa*. The resistance of *L. saligna* CGN 5271 was found to be based on a *Dm* like resistance gene, *R39*, and three QTLs (Jeuken and Lindhout in press). To extend our knowledge about the genetic basis of non-host resistance of *L. saligna* to *Bremia*, we compared the genetics of non-host resistance to *Bremia* of another *L. saligna* accession with that in *L. saligna* CGN 5271.

The main aims of the present research with a new *L. saligna* accession were: 1) Generate a genetic map of an F_2 population from a cross between this *L. saligna* accession and a *L. sativa* cultivar 2) Map QTLs involved in quantitative resistance to *Bremia* by performing a disease test and QTL mapping on the F_2 population 3) Generate a set of BILs by backcrossing. Our specific goal was to develop two types of BILs, both with an introgression of the new *L. saligna* accession. In one type of BILs the introgression covered the resistance gene(s) detected in the F_2 population from the new *L. saligna* accession. In the other type of BILs the introgression would cover genome regions with potential resistance genes that were detected in the F_2 population and BILs based on *L. saligna* CGN 5271. The results of the first goal, a genetic map, were described in the integrated AFLP map of lettuce (Jeuken et al. 2001). The results of goals 2 and 3 are described in this chapter.

MATERIAL AND METHODS

Plant materials and development of Backcross Inbred Lines

Three F_2 populations of crosses between *L. sativa* and three different *L. saligna* accessions were available. The F_2 populations from *L. saligna* CGN 5327, CGN 4662 and CGN 11341 had a population size of respectively 51, 34 and 54 F_2 plants. Based on the criterion of the largest F_2 population, *L. saligna* CGN 11341 was chosen for this study.

An F_2 population of 54 plants and a BC_1 population of 15 plants derived from the same single F_1 plant were grown in the greenhouse under standard conditions. The parents of the F_1 were *L. saligna* CGN 11341 as female parent and *L. sativa* cv "Norden", a butterhead cultivar, as male parent (designated as population B in Jeuken et al. 2001). *L. sativa* Norden contained the known *Bremia* resistance genes *Dm*3 and *Dm*11 and was susceptible to *Bremia* NL14 and NL16 (Jeuken and Lindhout in press).

L. sativa Norden was the male parent to generate the BC_1. The BC_1 generation was crossed reciprocally with *L. sativa* Norden to obtain the BC_2. BC_2 and BC_3 plants were backcrossed with *L. sativa* Norden as a mother to obtain the BC_3 and BC_4 respectively. From the BC_1 and BC_3 generations we analyzed 15 and 42 individuals with 145 and 133 AFLP markers respectively. According to the genetic map of *L. saligna* × *L. sativa* (Jeuken et al. 2001), graphical genotypes were made from each BC_1 and BC_3 plant using the software program Graphical GenoTyping (GGT, Van Berloo 1999, http://www.dpw.wau.nl/pv/pub/ggt).

Disease test

Two disease tests with *Bremia* race NL16 were performed on 7 and 8.5 weeks old F_2 and BC_1 plants. One disease test with race NL14 was performed at 7 weeks old F_2 and BC_1 plants. Disease tests were performed and scored as described for F_2 plants of Population A in Jeuken et al (2001) with the exception that one replication of three leaf discs were used per plant.

QTL mapping

A QTL mapping procedure was performed on the F_2 population of 54 plants. The software program MapQTL 4.0 (Van Ooijen and Maliepaard, 1996) was used to perform the Kruskal-Wallis tests and the Interval Mapping method on the average infection severity scores per plant of the two disease tests with *Bremia* NL16 (based on observations on six leaf discs) and on the average infection severity score per plant of the single disease test with NL14 (based on observations on three leaf discs). The complete DNA marker data set of 223 markers was used in the Kruskal-Wallis test and the Interval Mapping (Jeuken et al 2001).

The criteria for detecting a QTL were set by a significance level of 0.005 in the Kruskal–Wallis test. For the Restricted MQM method (no dominance fitted) a LOD threshold value of 2.9 was used (Van Ooijen, 1999).

RESULTS

Bremia disease tests

The F_2 population showed a wide range in infection severity scores from completely resistant (scale value 0) to completely susceptible (scale value 4) to both *Bremia* races (Figure 1a). The susceptible parent *L. sativa* Norden showed an infection severity score of 4.0 to NL14 as well as to NL16. The resistant parent *L. saligna* CGN 11341 showed an infection severity score of 0.0 to NL14 and NL16. The heritability for *Bremia* resistance was 0.81 in the first test and 0.92 for the second test with NL16 and 0.81 in the test with NL14. These high heritabilities indicate a high genetic variation and a small error in these disease tests on the F_2 and BC_1 plants.

The Pearson correlation coefficient between two tests with NL16 race was 0.87. So results with *Bremia* race NL16 were reproducible. To present the results of the tests with different *Bremia* races, we averaged the infection severity scores of F_2 and BC_1 plants over both tests with NL16. In this way, the number of missing values of plant and/or leaf discs observations was minimal. Because of the high correlation between disease tests with race NL16, we did not correct the data for experiment effects. The results of reactions to the two different *Bremia* races in the F_2 population were compared to study a possible race-specificity of the resistance (Figure 1a). The main body of the data points was scattered around the diagonal, indicating (a) a quantitative expression of resistance to both *Bremia* races and (b) similar level of resistance to both races. In addition to this general picture, a small number of F_2 plants showed

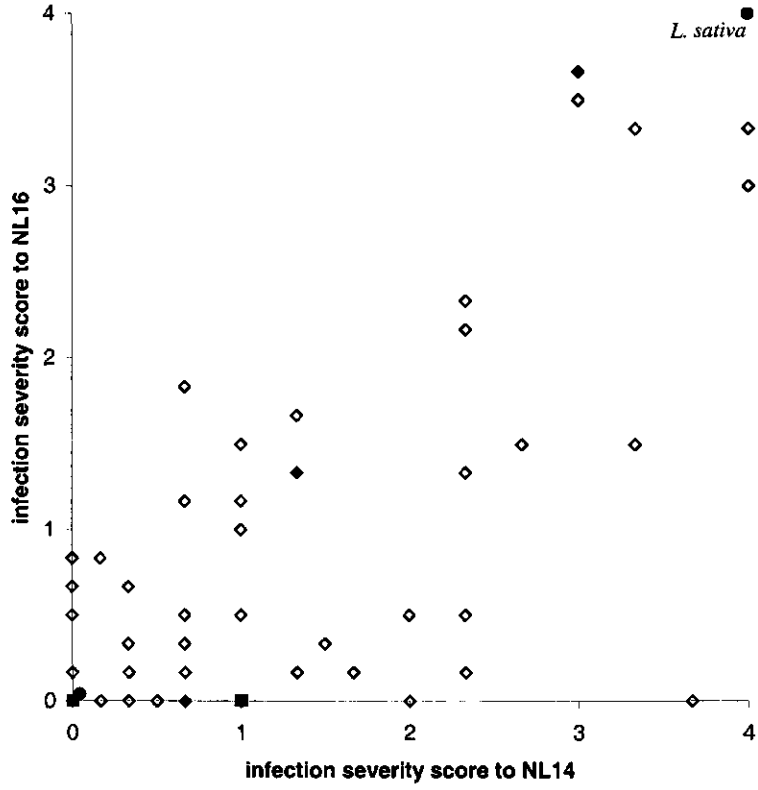


FIGURE 1a. Comparison of resistance against *Bremia* races NL14 and NL16 in the F_2 between *L. saligna* CGN 11341 and *L. sativa* Norden (Figure1a) and in the BC_1 (Figure1b). The average infection severity scores (diamond and square symbols) are shown per plant based on observations on maximally six leaf discs in two experiments. *L. sativa* Norden is the susceptible control (9 observations for each *Bremia* race) and *L. saligna* CGN 11341 is the resistant control (9 and 18 observations for NL14 and NL16 respectively; black dot symbols). Infection severity scores: class 0: no sporulation, class 1: 1-25% of leaf disc area sporulates, class 2: 26-50%, class 3: 50-75% and class 4: 75-100%. Black diamonds and black squares show the infection severity scores represented by two and five plants.

clear dissimilar levels of resistance to races NL14 and NL16, which points towards race-specificity. Apart from a shift towards an overall higher level of resistance in the F_2 population, the same pattern is observed in the BC_1, where the outliers in the scatter diagram are more pronounced than in the F_2 (Figure 1b). Taken together, the results of the F_2 and BC_1 disease tests indicate (a) an overall quantitative race-non-specific nature of the resistance and (b) the segregation of race-specific resistance to NL16.

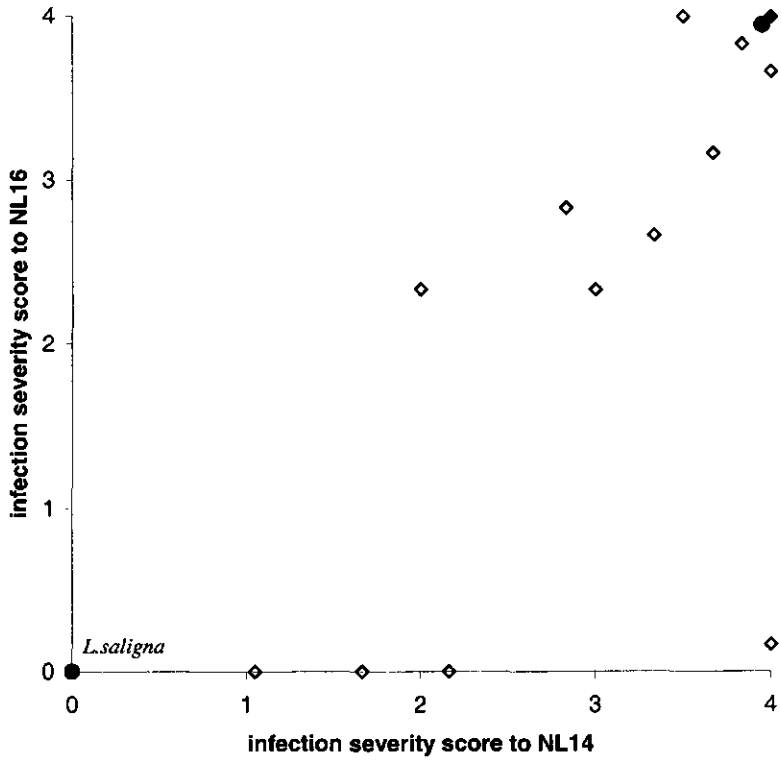


FIGURE 1b. Legends see Figure 1a

Mapping of *Bremia* resistance

The 54 F_2 plants were genotyped with 223 AFLP markers with known positions on the integrated linkage map (Jeuken et al. 2001). This population size is too small for reliable QTL mapping and we expected to detect major genes only. The small population size was due to the low seed set and the low germination rate of 42%. One QTL was detected in the F_2 population (Table 1). This QTL confers resistance to *Bremia* NL16, but not to NL14. The QTL was closely linked with the AFLP marker E54M48-264sal, which was mapped on the integrated lettuce map on Chromosome 9 at 3 cM (Jeuken et al. 2001). This is the same locus as the position of *R39* from *L. saligna* CGN 5271 (Jeuken and Lindhout in press). A comparison was made of average infection severity scores of F_2 and BC_1 plants, which were divided into classes containing no, one or two alleles of this detected QTL, based on the genotype of the nearest AFLP markers (Table 2). The pattern of infection severity scores to NL16 compared to NL14 indicated a race-specific effect of this QTL. One or two copies of the resistance alleles of the QTL gave an average reduction of the infection

TABLE 1. QTL for *Bremia* resistance in an F_2 population from *L. saligna* CGN 11341 × *L. sativa*. The QTL was identified if the threshold of the Kruskal-Wallis test or the Interval Mapping Method was exceeded.

QTL name	Chromosome number and QTL interval	*Bremia* race	Kruskal - Wallis test [a] significance	Restricted MQM mapping Fit dominance yes [b]		
				Peak LOD Score	Exp%	Add
R39b	9, 3 cM	NL16	xx	3.1	25	1.0

a
The Kruskal-Wallis test P- value = significance level
xx = 0.001
b
Exp% = proportion of the explained phenotypic variance.
Add = additive effect of the resistance allele.

severity against NL16 of about 1.5 in 38 F_2 plants. In the four BC_1 plants an even stronger reduction of 3.1 of just one allele of the QTL was observed for the test with *Bremia* NL16. Apparently, this gene is dominant. As the features of this gene resemble those of *R39* from *L. saligna* CGN 5271, we designated this gene *R39b*. A small and insignificant reduction of the infection severity in the test with NL14 was observed in the F_2 and the BC_1 for genotypes with one or two alleles of the QTL (Table 2).

The four QTLs (*RBQ1*, *RBQ2*, *RBQ3* and *RBQ4*) for *Bremia* resistance from *L. saligna* CGN 5271, were not detected by QTL mapping in this F_2 population.

However, when average infection severities are calculated from genotype classes of these four QTLs, F_2 plants with *L. saligna* AFLP marker alleles at the QTL loci showed indeed a lower infection severity than with *L. sativa* alleles at the QTL loci (Table 3). This indicates that *L. saligna* CGN 11341 harbors QTLs for resistance to *Bremia* on the same chromosome regions as *L. saligna* CGN 5271. For *RBQ1* and

TABLE 2. Average infection severity score in disease class scale of 0-4, based on one test with NL14 and the average of two tests of NL16. F_2 and BC_1 plants were divided into genotype classes at the *R39b* locus (Chromosome 9 at 3 cM).

	R39b locus*	# plants	NL14 [κ]	NL16 [κ]
F_2	a	12	1.97^{cd}	2.11^{c}
	h	33	1.25^{c}	0.58^{d}
	b	5	0.87^{c}	0.47^{d}
BC_1	a	10	3.42^{d}	3.28^{c}
	h	4	2.21^{cd}	0.04^{d}

[κ] Letters in common within a column, indicate that the values are not significantly different (α =0.05, Tukey HSD procedure)
* a= homozygous *L. sativa*
h= heterozygous
b= homozygous *L. saligna*

RBQ2 the differences between resistance and susceptibility alleles were even statistically significant.

Backcross program

Backcrosses were made starting from the BC_1 generation to the BC_4 generation. All backcrosses from F_1 to the BC_4 were made randomly without selection on genotype, because at that time a major requisite, a linkage map of *L. saligna* × *L. sativa*, was not available yet. The BC_1 and BC_3 were genotyped after an integrated linkage map of lettuce was constructed (Jeuken et al 2001). In ten BC_1 plants that were used in the backcross program, at least 96% of the genome of *L. saligna* was represented. The remaining 4% of the genome was not sufficiently covered by AFLP markers to analyze the genotypes of the BC_1 plants in that region (bottom part of Chromosome 1). In 19 BC_3 plants that were used to generate the BC_4 generation, 71% of the genome of *L. saligna* was present, 7% of the *L. saligna* genome was absent and 22% of the genome was not genotyped because of the absence of AFLP markers in that region. In three chromosome regions no *L. saligna*-derived markers were recovered in the selection of 19 BC_3 plants: Chromosome 5 from 70 to 92 cM, Chromosome 7 from 25 to 45 cM and Chromosome 9 from 0 to 16 cM. Unfortunately, *R39b*, located on the top of Chromosome 9, was not present in any BC_3 plant. To retrieve the *L. saligna* introgression with *R39b*, backcrosses were made on a BC_1S_1 plant that still harbored this introgression. The chromosomal regions of introgressions with other potential resistance genes, mapped as resistance genes in *L. saligna* CGN 5271 (*RBQ1*, *RBQ2*, *RBQ3* and *RBQ4*), were all still present in the selection of 19 BC_3 plants.

TABLE 3. The average infection severity of F_2 plants of *L. saligna* CGN 11341 × *L. sativa* Norden to race NL14 of *Bremia lactucae* is shown (in classes from 0 to 4 that are resistant to susceptible). The F_2 plants are classified according the genotype for potential loci of QTLs. These QTLs (*RBQ1* to *RBQ4*) are mapped in *L. saligna* CGN 5271.

NL14 disease test	*RBQ1*		*RBQ2*		*RBQ3*		*RBQ4*	
genotype at *RBQ* locus*	average infection severity [κ]	# of plants	average infection severity [κ]	# of plants	average infection severity [κ]	# of plants	average infection severity [κ]	# of plants
a	2.27[c]	11	2.28[c]	9	1.64[c]	18	1.44[c]	9
h	1.27[d]	24	1.22[d]	29	1.37[c]	23	1.48[c]	28
b	0.91[d]	15	1.10[d]	12	0.90[c]	8	1.14[c]	13
u	N.D.	4	N.D.	4	N.D.	5	N.D.	4

[κ] Letters in common within a column, indicate that the values are not significantly different (α =0.05, Tukey HSD procedure)

* a =homozygous *L. sativa*
h= heterozygous
b =homozygous *L. saligna*
u = no genotype data available

been made between tomato (*Lycopersicon esculentum*) and *Solanum lycopersicoides*, the most distant crossable wild relative of cultivated tomato (Rick 1979). This tomato study and the *L. saligna* × *L. sativa* study in this thesis, are one of the few studies on hybridization between highly polymorphic parents that are continued by further backcrossing resulting in a set of Backcross Inbred Lines (Chetelat and Meglic 2000, Chapter 4). They share several similar results typical for hybridization of genetically remote species like: a) Chromosomal rearrangements, such as inversions or translocations, are not observed. b) Segregation distortion is often observed. c) Often sterility is observed that is mostly explained by epistasis.

Taxonomical studies have suggested that *L. sativa* has been domesticated from the direct ancestor *L. serriola*, with probably involvement of one or two more wild lettuce species, *L. saligna* and *L. virosa* (Hill et al 1996, de Vries 1997, Koopman et al 2001). For a speculation about speciation and reproductive barriers in the *Lactuca* species and the influence of *L. saligna* in domestication of lettuce, it is interesting to assume the possibility of a natural hybridization between *L. saligna* CGN 5271 and *L. sativa* Olof and to speculate about the consequences of it. Assume a natural hybridization between these two species in a field full of *L. sativa* Olof plants and one *L. saligna* CGN 5271 plant (like an uncontrolled hybridization in farmers' lettuce field). What kind of hybrid species with which *L. saligna* introgressions would be expected after several generations of mostly selfings and some backcrosses with *L. sativa* Olof (outcrossing)? Based on our observations the following expectations can be formulated. A species mostly related to *L. sativa* with some minor *L. saligna* introgressions is expected due to many skewed segregation ratios favoring *L. sativa* alleles. The natural selection and segregation distortion would determine which chromosome segments of *L. saligna* would finally be maintained in this population. The most likely introgressed *L. saligna* segment would be the top of Chromosome 5 as it showed a high preference for *L. saligna* alleles (allele frequency of 0.76 in F_2 and 0.95 in advanced backcross line with *L. sativa* as mother, Chapter 4). When selection for *Bremia* resistance would have occurred during this process, alleles of resistance genes from *L. saligna* would have had a chance to become fixed. However, this chance would have been rather small, as most resistance loci were in regions with severe distorted segregation favoring *L. sativa* alleles and excluding genotypes with homozygous resistance alleles of *L. saligna* (*R39*, *RBQ1* and specifically *RBQ2* in backcross lines). Alternatively, one resistance locus was linked to extreme not favorable phenotypes (*RBQ3* linked with an aberrant leaf surface and pigmentation, which probably has a lower fitness). Only *RBQ4* would have had a chance for natural introgression.

Conclusion: The success of introgression of resistance genes in this hypothesized "natural hybridization" between two remote species is low, mainly due to severe distorted segregations in hybrids.

COMPARISON OF BREEDING METHODS

Conventional breeding methods make use of selections on phenotypic traits. This is relatively simple for monogenic inherited, easy recognizable traits, but much harder for quantitatively expressed or polygenically inherited traits like partial or non-host resistance or yield. Modern breeding methods may select on phenotype and on genotype by using Marker Assisted Selection (MAS). When MAS is used, genotypes are selected for a diagnostic DNA marker that is closely linked to the desired phenotype.

For each specific breeding goal in a crop, the breeder has to decide which breeding method is most efficient. Breeding methods can differ in selection criterion like phenotype or genotype and in population type to select from like F_2 or backcross populations. MAS has not been used intensively in lettuce breeding yet, since the limited availability of high throughput marker technologies and of information on diagnostic PCR-markers linked to traits. However, molecular genetic information in lettuce is rapidly accumulating, enabling breeders to use MAS.

The data in this thesis are perfect for a study on the efficiency of various selection methods with as variables population type (F_2/F_3 and advanced breeding lines like BILs) and the selection tool (MAS and/or phenotype selection). To perform such a study, sampling of populations from a population of infinite size or from more accessions would be ideal. However, our data are not chosen from an unlimited number of independent subsets, but from discrete populations from discrete parents. Nevertheless, our genetic results are still very useful to get insight in the efficiencies of different breeding methods.

With our data the results of four different methods can be compared (Figure 2). These four breeding methods, determined by resistance detection in two population types and by use of two selection tools, will be evaluated for the chance that a specific resistance gene is recovered in advanced breeding lines (Table 1).

R39 is a *Dm* like dominant resistance gene that gives complete race–specifc resistance. It is mapped at the top of Chromosome 9 and is closely linked to partial male sterility (Chapter 2 and 3). *R39* would have been detected in all four breeding methods except in method C in which the BC_4S_1 is developed without MAS. The introgression of the top of Chromosome 9 would have been lost during four backcross generations, due to a distorted segregation favoring *L. sativa* alleles caused by partial male sterility closely linked to *R39*. For the breeding success of *R39* method D would be most successful, while the efficiency of method A and B would depend on the availability of fertile F_2 plants with *R39*.

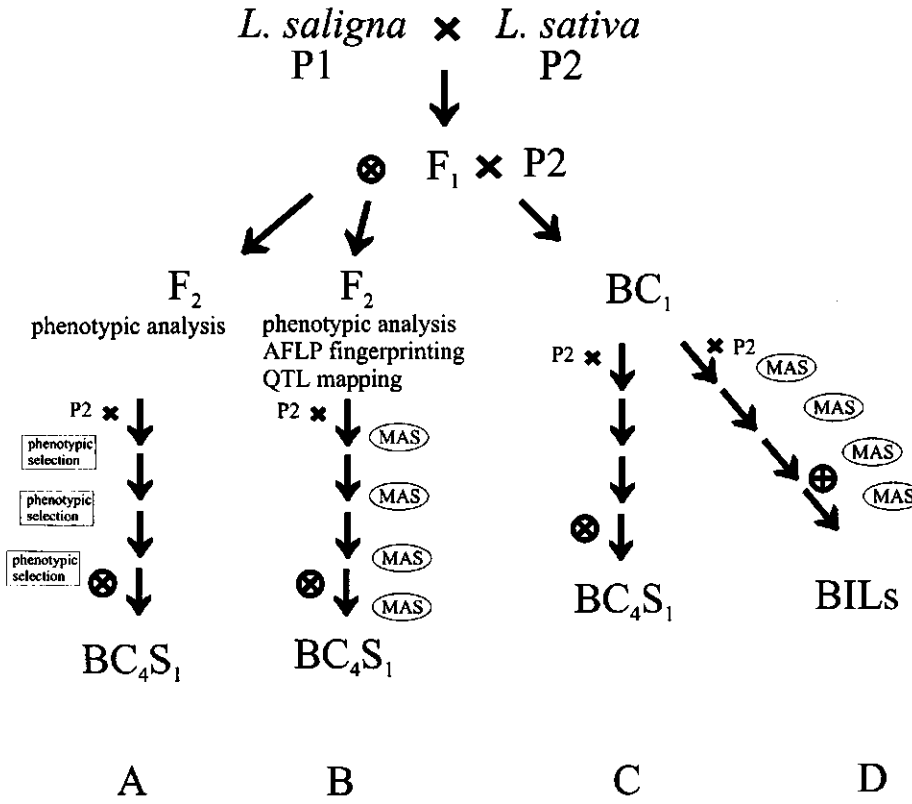


FIGURE 2. The four breeding methods (A, B, C and D) are compared for efficiency in introgression of *Bremia* resistance genes from *L. saligna* in cultivated lettuce. MAS = Marker Assisted Selection. At the end stage of each breeding method a phenotypic analysis is performed to detect the introgressed resistance genes. Methods: (A) an F_2 population that is phenotypically tested by a *Bremia* disease test. Further introgression of the detected resistance is performed by phenotypic selection during repeated backcrossing and one selfing till the BC_4S_1 generation (B) an F_2 population that is phenotypically and genotypically tested, enabling QTL mapping (Chapter 2 and 3). Further introgression of the detected resistance is performed by repeated backcrossing and one selfing using MAS till the BC_4S_1 generation. (C) a BC_4S_1 population, developed by randomly backcrossing and one selfing. It is only phenotypically tested for *Bremia* resistance at the BC_4S_1 stage (D) a set of BILs that is genotypically and phenotypically tested (Chapter 4 and 5). The BC_4S_1's and the BILs are considered as advanced breeding lines and are tested for the chance that resistance loci are recovered after the breeding program.

TABLE 1. The chance for introgression of resistance genes from *L. saligna* CGN 5271 to *Bremia lactucae* is compared by using four different breeding methods (See Figure 2).

Breeding method/strategy	A	B	C	D
use of DNA markers (MAS)	no	yes	no	yes
resistance locus	Breeding success of resistance locus			
R39	yes[b]	yes[b]	no[d]	yes
RBQ1	no[a]	yes[b]	no[d]	?[f]
RBQ2	no[a]	yes[b]	no[e]	no[e]
RBQ3	no[a]	yes[b]	yes	yes
RBQ4	no[c]	no[c]	yes	yes

[a] Phenotypic selection for one single QTL is difficult and mainly limits the introgression of the QTL. Additionally, skewness favoring *L. sativa* alleles (*RBQ1*) and the chance that the selected F_2 or F_3 are sterile and backcrosses fail make introgression virtually impossible (Jeuken et al 2001).

[b] Phenotypic or genotypic selection for the resistance gene is feasible and does not limit introgression. Only the chance that the selected F_2 or F_3 are sterile and backcrosses fail can limit or reduce the efficiency of introgression (Jeuken et al 2001).

[c] Detection and introgression of this QTL was not possible due to distorted segregation with a strong preference for heterozygotes and a slight preference for *L. sativa* alleles (Chapter 3 and 5).

[d] The chromosome region of this locus would be lost during backcrossing due to distorted segregations favoring *L. sativa* alleles (Chapter 4).

[e] This QTL would not have been detected and introgressed since no BIL with this QTL in homozygous state would occur. A plant with one allele of this resistance QTL would not have been detected as more resistant than a genotype without the resistance allele and hence cannot be selected for (Chapter 5).

[f] A BIL harboring this QTL has not been developed yet and therefore no conclusion can be drawn.

RBQ1 gives quantitative resistance to *Bremia*. It was mapped at Chromosome 7 in a region with a distorted segregation favoring *L. sativa* alleles (Chapter 2 and 3). *RBQ1* would have been detected and introgressed only when MAS was used. The use of phenotypic selection in the F_2 only would not allow successful detection and introgression of *RBQ1*, due to a combination of the quantitative effect of the resistance, the skewed segregation favoring *L. sativa* alleles and the probability of sterile F_2 plants. *RBQ1* would also not have been detected in the BC_4S_1 by phenotypic selection, since the introgression of *RBQ1* at Chromosome 7 would have been lost during four backcross generations due to the skewed segregation.

RBQ2 gives quantitative resistance to *Bremia* and was mapped at the top of Chromosome 1 (Chapter 2 and 3). *RBQ2* would only have been detected in the F_2 using DNA markers. A successful introgression of *RBQ2* in an advanced backcross line by this method B depends on the chance of obtaining a genotype with an introgression of *RBQ2* in homozygous state, which was not possible during BIL development (Chapter 4). Without the use of MAS, it would have been very difficult

or even impossible to introgress *RBQ2* due to the combination of the quantitative effect of the resistance and the probability of sterile F_2 plants. In the backcross populations (BIL and BC_4S_1), *RBQ2* would not have been detected since no introgression of the top of Chromosome 1 could be obtained homozygously (Chapter 4) and one *RBQ2* allele in heterozygous lines would not have exhibited resistance (Chapter 5).

RBQ3 gives quantitative resistance to *Bremia* and was mapped at Chromosome 9 (Chapter 3 and 5). *RBQ3* would have been detected and introgressed using all breeding methods except for the F_2 method without MAS, due to a combination of the quantitative effect of the resistance and the probability of sterile F_2 plants.

RBQ4 gives quantitative resistance to *Bremia* NL16. It was located on Chromosome 8 that had a distorted segregation in the F_2 with a strong preference for heterozygotes and a slight preference for *L. sativa* alleles (Chapter 2 and 5). *RBQ4* would not have been detected in an F_2 population regardless the use of DNA markers. This was mainly due to the severe distorted segregation of *RBQ4*. As a consequence, no effort would have been made to introgress this locus. *RBQ4* would have been detected in advanced breeding lines (Method C and D) regardless use of MAS, since BILs with a homozygous introgression for Chromosome 8 would occur and their resistance level would be high enough for detection. *RBQ4* would have been successfully introgressed by method C and D.

Summarizing, selection for resistance is expected to be more successful when MAS is used than without MAS, since in each method with use of MAS at least three resistance loci have been detected, whereas without use of MAS maximally one or two resistance loci are likely to be detected.

Comparison of introgression success per population type reveals that by use of only phenotypic selection an advanced breeding line (method C) is likely to introgress two QTLs, while an F_2 (method A) is likely to introgress only a major resistance gene. When MAS is also used for selection (in methods B and D) the same number of resistances are introgressed for both population types. So for the number of introgressed resistances in this study, the type of population used for detection doesn't show a large difference.

FUTURE RESEARCH AND RECOMMENDATIONS

The research described in this thesis is being continued for another two years. We will continue the development of a BIL population that nearly completely (97%) covers the genome of *L. saligna* CGN 5271. BILs harboring *RBQ3* and *RBQ4* will be crossed and selected for presence of both genes homozygously in one genotype. This line with two QTLs for resistance and all newly developed BILs will be tested for resistance to the *Bremia* races NL14 and NL16 by a leaf disc test. From these tests we will quantitatively measure the effect of *RBQ1*, maybe *RBQ2* and the effects of *RBQ3* and *RBQ4* expressed together in one genotype. Possibly, we will detect a new QTL for resistance not detected in the F_2. When all QTLs for resistance are detected in the BIL

population, the BILs harboring QTLs for resistance will be tested by leaf disc tests with 28 additional *Bremia* races to study race-specificity. Furthermore, these BILs with QTLs will be studied for genotypexenvironment interactions by phenotypic characterization of plants in six different environments. The variables for the environments are three locations, the Netherlands, France and Spain and two cultivation methods: in the greenhouse and in the field. Other experiments that will be considered are histological studies on BILs with resistance QTLs, in which we may observe the action and timing of resistance (with or without a role for the hypersensitive response?). An attempt to develop a susceptible *L. saligna*-like genotype will be made by continuous selfing and Marker Assisted Selection of the susceptible F_3 line (from F_2 plant 35 that harbors *L. saligna* alleles at 60% of the haploid genome, Chapter 3) that lacked resistance alleles of *R39*, *RBQ1*, *RBQ2*, *RBQ3* and segregated for resistance alleles at the *RBQ4* locus. This *L. saligna* –like genotype will be included in disease tests and histological tests.

L. saligna CGN 5271 will be screened for the presence of more unknown *Dm* genes by performing a disease test on seedlings of the complete set of BILs with 28 *Bremia* races of importance for the European lettuce growers (This is a quick screening that is less intensive and less expensive than disease tests on leaf discs). When it is known how many *Dm* genes are present in *L. saligna* CGN 5271 and with which resistance spectra, more information is gathered about the role of *Dm* genes in a non-host. Comparison of *Dm* genes from *L. saligna* with *Dm* genes from *L. sativa* concerning abundance, location and resistance spectra may reveal information about evolution of these resistance genes in a host and in a non-host.

The study on the resistance of *L. saligna* CGN 11341 compared to the resistance of the other *L. saligna* accession will be continued. BILs with *R39b* and genome regions homologous to the regions carrying resistance genes in *L. saligna* CGN 5271 will be developed and tested for *Bremia* resistance and analyzed histologically. This will give us more insights on the possible presence of a general resistance mechanism to *Bremia* within the *L. saligna* species.

Besides these planned experiments other studies are feasible in the future. Studies on gene expression related to downy mildew resistance would be very interesting to perform with resistant BILs. Interesting gene expressions to test would be the expression of pathogenesis related (PR) genes and genes associated with the hypersensitive response (HR). Profiling of expressed genes associated to resistance could be studied by a cDNA AFLP analysis on resistant BILs, exposed and not-exposed to downy mildew.

A large step forward in the uncovering of the non-host resistance mechanism and its signal pathway would be achieved by cloning the QTLs for resistance. To do so, a successful and efficient map based cloning strategy is required. Therefore, the following conditions need to be met. 1) A disease test that reveals clearly distinctive resistance difference between genotypes with and without the QTL. 2) Markers closely linked to the QTL (obtained by marker search on recombinants segregating for resistance originating from BILs) 3) a genomic library of *L. saligna* CGN 5271. Out of the four QTLs for resistance to *Bremia*, *RBQ4* has the best conditions for cloning at

this moment. *RBQ4* has the highest reduction on infection severity by *Bremia*. A BIL with *RBQ4* is available that does not show an undesirable phenotype (linkage drag).
The cloning of *R39* and *R39b* would be interesting for sequence comparison with the cloned *Dm3* gene of *L. sativa* and for mutual sequence comparison (Meyers et al 1998a). Similar to the described map based cloning strategy for *RBQ* genes, *R39* and *R39b* may be cloned. However, the presence of a DNA marker in the sequence of the resistance gene (complete cosegregation) could accelerate their cloning procedure. Such marker may be acquired by the use of specificly designed primers based on conservative regions in the *Dm3* sequence, supposing homology between *Dm* genes.
Resistance genes are not the only interesting and valuable traits that can be derived from the set of BILs. This set can be considered a genomic library for *L. saligna* and all its traits. Since the BILs are a permanent resource with defined introgressions, they can be used over time for detection and mapping of several traits in *L. sativa* and *L. saligna* for scientific or breeders' interest.
At the moment some research groups started a joint "Composite Genomics Project" in which 80.000 Expressed Sequence Tags (ESTs) of lettuce and sunflower are generated (publicly accessible in 2002 at http://compgenomics.ucdavis.edu/). The set of BILs with *L. saligna* introgressions would be an ideal mapping population to map the lettuce ESTs and maybe the sunflower ESTs. Later, EST-derived PCR markers may be useful in comparative mapping between Compositae species. At the moment Compositae linkage maps exist for lettuce (Jeuken et al 2001), chicory (n=9; De Simone et al 1997), sunflower (n=17; Gedil-Melaku et al 2001) and *Tetramolopium* (n=9; Whitkus 1998). These species and dandelion (*Taraxum officinale* L., n=8) that harbors the interesting trait natural apomixus, may be used for comparative mapping studies. Besides the use of ESTs for genetic mapping, these lettuce ESTs may facilitate gene expression studies when combined with DNA microarrays.
In conclusion, the research in this Ph.D. thesis provides a solid basis for fundamental studies on *L. saligna* and non-host resistance in the future. Further, it adds to the general scientific knowledge on lettuce and this may also be of help for studies in Compositae species that show syntheny with lettuce.

ACKNOWLEDGEMENT

P. Stam and R.E. Niks are thanked for critically reading the manuscript.

REFERENCES

Agrios GN (1997) Plant Pathology, 4th edn. Academic Press, San Diego

Arumuganathan K and Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208-218

Bernatsky R, Tanksley SD (1989) Restriction fragments as molecular markers for germplasm evaluation and utilisation. In: AHD Brown et al. (eds) The use of plant genetic resources. Cambridge University Press, Cambridge pp 353-362

Bonnier FJM, Reinink K and Groenwold R (1992) New sources of major gene resistance in *Lactuca* to *Bremia lactucae*. Euphytica 61:203-211

Bonnier FJM, Reinink K and Groenwold R (1994) Genetic analysis of *Lactuca* accessions with new major gene resistance to lettuce downy mildew. Phytopathology 84:462-468

Botella MA, Parker JE, Frost LN, Bittner EPD, Beynon JL, Daniels MJ, Holub EB and Jones JDG (1998) Three genes of the arabidopsis *RPP1* complex resistance locus recognize distinct *Peronospora parasitica* avirulence determinants. Plant Cell 10:847-1860

Boukema IW, Hazekamp T and van Hintum TJL (1990) CGN Collection Reviews: the CGN Lettuce Collection. Centre for Genetic Resources (CGN), Wageningen, the Netherlands

Campbell NA (1993) Biology, 3rd edn. The Benjamin/Cummings Publishing Compagny

Chetelat RT and Meglic V (2000) Molecular mapping of chromosome segments introgressed from *Solanum lycopersicoides* into cultivated tomato (*Lycopersicon esculentum*). Theor Appl Genet 100:232-241

Chetelat RT, Meglic V and Cisneros P (2000) A genetic map of tomato based on BC_1 *Lycopersicon esculentum* × *Solanum lycopersicoides* reveals overall synteny but suppressed recombination between these homeologous genomes. Genetics 154:857-867

Chittenden LM, Schertz KF, Lin YR, Wing RA, and Paterson AH (1994) A detailed RFLP map of *Sorghum bicolor* × *S. propinquum*, suitable for high-density mapping, suggests ancestral duplication of *Sorghum* chromosomes or chromosomal segments. Theor Appl Genet 87:925-933

Cooley MB, Pathirana S, Wu HJ, Kachroo P and Klessig DF (2000) Members of the *Arabidopsis HRT/RPP8* family of resistance genes confer resistance to both viral and oomycete pathogens, Plant Cell 12:663-676

Crute IR (1992) From breeding to cloning (and back again?): a case study with lettuce downy mildew. Annu Rev Phytopathol 30:485-506

Crute IR and Johnson AG (1976) The genetic relationship between races of *Bremia lactucae* and cultivars of *Lactuca sativa*. Ann Appl Biol 89:476-474
Crute IR and Norwood JM (1981) The identification and characteristics of field resistance to lettuce downy mildew (*Bremia lactucae* Regel). Euphytica 30:707-717

De Simone M, Morgante M, Lucchin M, Parrini P and Marocco A (1997) A first linkage map of *Cichorium intybus* L. using a one-way pseudo-testcross and PCR-derived markers. Mol Breeding 3:415-425

Tosa Y and Shishiyama J (1984) Defense reactions of barley cultivars to an inappropriate forma speciales of the powdery mildew fungus of gramineous plants. Can J Bot 62:2114-2117

Van Berloo R (1999) The Development of software for the graphical representation and filtering of molecular marker data: graphical genotypes (GGT), J Hered 90:328-329

Van de Peer Y and De Wachter R (1997) Evolutionairy relationships among the eukaryotic crown taxa taking into account site-to-site rate variation in 18S rRNA. J Mol Evol 45:619-630

Van de Wiel CCM, Arens P and Vosman B (1999) Microsatellite retrieval in lettuce (*Lactuca sativa* L.). Genome 42:139-149

Van der Beek JG, Verkerk R, Zabel P and Lindhout P (1992) Mapping strategy for resistance genes in tomato based on RFLPs between cultivars: *Cf-9* (resistance to *Cladosporium fulvum*) on chromosome 1. Theor Appl Genet 84: 106-112

Van Eck HJ, Rouppe van de Voort J, Draaistra J, Van Zandvoort P, Van Enckevort E, Segers B, Peleman J, Jacobsen E, Helder J and Bakker J (1995) The inheritance and chromosomal localization of AFLP markers in a non-inbred potato offspring. Mol Breeding 1:397-410

Van Ettekoven K & van der Arend AJM, (1999) Identification and denomination of "new"races of *Bremia lactucae*, In: Lebeda A and Křístková E (eds) Proc. EUCARPIA Meeting on Leafy Vegetables, Olomouc, Czech Republic pp 171-181

Van Heusden AW, van Ooijen JW, Vrielink-van Ginkel R, Verbeek WHJ, Wietsma WA and Kik C (2000) A genetic map of an interspecific cross in *Allium* based on amplified fragment length polymorphism (AFLP ™) markers. Theor Appl Genet 100: 118-126

Van Ooyen JW (1999) LOD significance thresholds for QTL analysis in experimental populations of diploid species. Hered 83, 613-624

Van Ooyen JW and Maliepaard C (1996) MapQTL (™)version 4.0: Software for the calculation of QTL positions on genetic maps. CPRO-DLO, Wageningen, The Netherlands

Van Wees SCM, de Swart EAM, van Pelt JA, van Loon LC and Pieterse CMJ (2000) Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis thaliana* P Natl Acad Sci USA 97:8711-8716

Vleeshouwers VGAA, van Dooijeweert W, Govers F, Kamoun S and Colon LT (2000) The hypersensitive response is associated with host and nonhost resistance to *Phytophthora infestans*. Planta 210, 853-864

Vos P, Hogers R, Bleeker M, Reijans M, Van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M and Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23: 4407-4414

Vuylsteke M, Mank R, Antonise R, Bastiaans E, Senior ML, Stuber CW, Melchinger AE, Lübberstedt T, Xia XC, Stam P, Zabeau M and Kuiper M (1999) Two high-density AFLP™ linkage maps of *Zea mays* L.: analysis of distribution of AFLP markers. Theor Appl Genet 99: 921-935

Whalen MC, Stall RE and Staskawicz BJ (1988) Characterization of a gene from a tomato pathogen determining hypersensitive resistance in non-host species and genetical analysis of this resistance in bean. P Natl Acad Sci USA 85:6743-6747

Whitkus R (1998) Genetics of adaptive radiation in Hawaiian and Cook Islands species of *Tetramolopium* (*Asteraceae*). II. Genetic linkage map and its implications for interspecific breeding barriers. Genetics 150:1209-1216

Witsenboer H, Kesseli RV, Fortin MG, Stanghellini M and Michelmore RW (1995) Sources and genetic structure of a cluster of genes for resistance to three pathogens in lettuce. Theor Appl Genet 91:178-188

Young ND (1996) QTL mapping and quantitative disease resistance in plants.Ann Rev Phytopathology 34:479-501

Young WP, Schupp JM, Keim P (1999) DNA methylation and AFLP marker distribution in the soybean genome. Theor Appl Genet 99:785-792

Zamir D (2001) Improving plant breeding with exotic genetic libraries. Nature Rev Genetics 2:983-989

Zhang HS, De la Rosa R, Rubiales D, Lubbers HH, Molenveld JW and Niks RE (1994) Role of partial resistance to *Puccinia hordei* in barley in the defence of barley to inappropriate rust fungi. Physiol Mol Plant Plathol 45:219-228

SUMMARY

Plants are continuously exposed to a wide variety of pathogens. However, all plant species are non-hosts for the majority of the potential plant pathogens. The genetic dissection of non-host resistance is hampered by the lack of segregating population from crosses between host and non-host species, since hardly any non-host is crossable with a host. We have studied the non-host resistance in *Lactuca saligna* (wild lettuce) to lettuce downy mildew (*Bremia lactucae*). *L. saligna* is one of the few examples of a non-host species that is crossable with a related host species, *L. sativa* (lettuce). Based on this interspecific cross, segregating populations have been developed for genetical analysis of the non-host resistance. To map the resistance, we have used two strategies in which we make use of DNA markers to genotype plants. As no accurate linkage map was available for lettuce, we started with the construction of a linkage map of *L. saligna* × *L. sativa*. In Chapter 2, the development of an integrated linkage map, based on two populations, is described. To acquire DNA markers, AFLP analyses have been performed on the F_2 populations of the crosses *L. saligna* CGN 5271 × *L. sativa* Olof and *L. saligna* CGN 11341 × *L. sativa* Norden. Based on these AFLP analyses the polymorphism rate between *L. saligna* and *L. sativa* is estimated to be 81%. A linkage map was constructed that comprises 12 SSRs and 476 AFLP markers over 854 cM in nine linkage groups (n=9). Since the markers are randomly spread over all chromosomes, we assume this map is an accurate representative of both parental genomes and very useful for Marker Assisted Selection.

The first mapping strategy for downy mildew resistance is described in Chapter 3. In that study, we have performed a QTL analysis on 126 F_2 plants of a cross between the resistant *L. saligna* CGN 5271 and the susceptible *L. sativa* Olof. For this QTL analysis all 126 F_2 plants have been tested for resistance in four disease tests with two complex *Bremia* races (NL14 and NL16). The F_2 population showed a wide and continuous range of resistance levels from completely resistant to completely susceptible. Evidence is presented for a quantitative resistance against both *Bremia* races as well as for a race-specific resistance against *Bremia* race NL16 and not against NL14. These disease test data sets have been combined with DNA marker data of all 126 F_2 plants that had already been obtained for the construction of the linkage map. QTL mapping revealed a qualitative gene (*R39*) explaining the race-specific resistance and three QTLs (*RBQ1*, *RBQ2* and *RBQ3*) explaining the quantitative resistance. The qualitative gene *R39* is a dominant gene that gives nearly complete resistance to race NL16 in *L. saligna* CGN 5271 and therefore it shows features similar to *Dm* genes (dominant race specific genes that give a complete resistance to downy mildew). The three QTLs explain 51% of the quantitative resistance against NL14, which indicates that probably not all QTLs have been detected in this F_2 population.

In addition to this rather classical F_2 mapping strategy, we have performed an alternative mapping strategy based on the development and characterization of a set of

Backcross Inbred Lines (BILs). These BILs are genetically nearly completely like *L. sativa* but contain a single chromosome substitution segment of *L. saligna* CGN 5271 (Chapter 4). Starting from an F_1 plant, BILs have been developed by four to five generations of backcrosses and one generation of selfing. All backcrosses from F_1 to BC_4 were made randomly without intentional selection. Marker Assisted Selection was started in the BC_4 generation. Finally, a set of 29 lines was obtained that covers 95% of the *L. saligna* genome, comprising 16 lines with a single homozygous introgression (BILs), one line with two homozygous introgressions, five lines with heterozygous single introgressions and seven lines with two or more heterozygous introgressions. Several chromosome regions showed severe distorted segregation in the F_2 population. Based on segregation ratios in backcross lines, we were able to explain distorted segregations of three chromosome regions observed in the F_2 population by genetic loci that are involved in pollen- or egg cell fitness.

When seed of the first developed BILs was available, a disease test had been set up to test if the BILs, which carried QTLs as identified in the F_2 population, showed enhanced levels of quantitative resistance indeed. Nine BILs (or nearly-BILs) have been tested for resistance to *Bremia* race NL16. They covered together 31% of the *L. saligna* parental genome. Two resistance loci detected in the F_2 population (*R39* and *RBQ3*) have been confirmed in the disease test on the BILs. *R39* is a dominant gene, which gives a complete resistance against *Bremia* race NL16. *RBQ3* reduces the infection severity of the susceptible *L. sativa* by 49% ten days post inoculation. The quantitative effects from the resistance genes in these BILs were higher than expected from the F_2 mapping results. No conclusive comparisons of *RBQ2* could have been made, as the introgression in the backcross line was not homozygous. *RBQ1* has not been tested. Most exciting, the BIL method revealed a new resistance locus on Chromosome 8 with a 77% reduction on the infection severity compared to the susceptible control ten days post inoculation. We conclude that the BIL mapping method can reveal new QTLs unnoticed in the F_2 mapping method and it enables a quantification of the resistance gene effect in a *L. sativa* background.

To extend our knowledge about the non-host resistance of *L. saligna* to *Bremia*, we have compared the genetics of non-host resistance to *Bremia* in *L. saligna* CGN 5271 with another accession *L. saligna* CGN 11341. The two accessions show a 39% AFLP polymorphism rate. We have analyzed the non-host resistance of *L. saligna* CGN 11341 by disease tests and DNA marker analyses on an F_2 and BC_1 population. Disease tests with *Bremia* races NL14 and NL16 showed a wide range of infection severity scores from resistant to susceptible to both races. The majority of plants had a similar resistance level to both *Bremia* races. These findings imply that the resistance of *L. saligna* is quantitatively expressed and is probably race non-specific. A few F_2 and BC_1 plants were completely resistant against *Bremia* race NL16 and rather susceptible to race NL14. QTL mapping revealed that a major resistance gene that was located on Chromosome 9 explains this race-specific resistance. This gene is designated *R39b*, as it may be different from *R39*.

No additional QTLs have been detected in this small F_2 population (n= 54). However, F_2 plants with *L. saligna* CGN 11341 alleles at loci of *RBQ1*, *RBQ2*, *RBQ3* and *RBQ4*

mapped in *L. saligna* CGN 5271, were more resistant than F_2 plants with *L. sativa* alleles at these loci. In conclusion, we state that it is very likely that the same genes explain the resistances to *Bremia* in both *L. saligna* accessions. A backcross program for a set of Backcross Inbred Lines (BIL) that cover *R39b* and loci for putative QTLs, is in progress.

In the last chapter of this thesis the basic results of the study have been discussed. We adduce that non-host resistance in *L. saligna* is not explained by accumulation of race-specific major resistance genes (*Dm* genes) but by a resistance mechanism based on QTLs. Further, we have made a comparison for efficiency of four breeding methods to introgress the resistance genes from *L. saligna*. Based on this study, we conclude that twice as many resistance genes are introgressed when Marker Assisted Selection is used. Finally, several recommendations concerning research on non-host resistance and the applications of Backcross Inbred Lines have been suggested.

SAMENVATTING

Planten worden voortdurend blootgesteld aan vele soorten pathogenen (ziekteverwekkers). Echter alle plantensoorten zijn voor de meeste potentiële plant-pathogenen geen waard (gastheer). De genetische analyse van niet-waard resistentie wordt gehinderd door het gebrek aan splitsende populaties afkomstig van kruisingen tussen waard en niet-waard plantensoorten, omdat deze bijna nooit kruisbaar zijn. Wij hebben de niet-waard resistentie bestudeerd van *Lactuca saligna* (wilde sla) tegen valse meeldauw (*Bremia lactucae*). *L. saligna* is een van de weinige voorbeelden van een niet-waard soort die kruisbaar is met een verwante waard soort, *L. sativa* (sla). Gebaseerd op deze kruising tussen twee soorten, zijn splitsende populaties ontwikkeld voor de genetische analyse van de niet-waard resistentie. Voor het karteren van de resistentie op een koppelingskaart van sla zijn twee strategieën toegepast, die beiden gebruik maken van DNA-merkers om het genotype van de planten te bepalen. Omdat er nog geen koppelingskaart voor sla beschikbaar was, hebben we een koppelingskaart ontwikkeld gebaseerd op de kruising *L. saligna* × *L. sativa*. In Hoofdstuk 2 is de ontwikkeling van een geïntegreerde koppelingskaart beschreven. Voor het verkrijgen van DNA-merkers, zijn AFLP analyses uitgevoerd op twee F_2 populaties van de volgende kruisingen: *L. saligna* CGN 5271 × *L. sativa* Olof en *L. saligna* CGN 11341 × *L. sativa* Norden. Uit deze analyses is de polymorfie verhouding tussen *L. saligna* and *L. sativa* geschat op 81%. Een geïntegreerde koppelingskaart van sla is ontwikkeld die 12 microsatellieten en 476 AFLP-merkers bevat, verspreid over 854 cM en negen koppelingsgroepen (n=9). Omdat de DNA-merkers willekeurig verspreid zijn over alle chromosomen, nemen wij aan dat deze koppelingskaart een accurate afspiegeling is van beide ouderlijke genomen en zeer geschikt is voor gebruik bij merkergestuurde selectie.

De eerste karteringsstrategie voor de resistentie tegen *Bremia* is beschreven in Hoofdstuk 3. In dat onderzoek hebben we een QTL analyse uitgevoerd op 126 F_2 planten afkomstig van een kruising tussen de resistente *L. saligna* CGN 5271 en de vatbare *L. sativa* Olof. Voor deze analyse zijn alle 126 F_2 planten getest op resistentie in vier ziektetoetsen met twee complexe fysio's van *Bremia* (NL14 en NL16). De F_2 populatie toonde een brede en continue reikwijdte aan resistentie-niveau's van volledig resistent tot volledig vatbaar. Bewijs was geleverd voor zowel een kwantitatieve resistentie tegen beide *Bremia* fysio's als voor een fysiospecifieke resistentie tegen *Bremia* fysio NL16 en niet tegen NL14. De gegevens van deze ziektetoetsen zijn gecombineerd met de gegevens van de DNA-merkers van alle 126 F_2 planten, die al verkregen waren voor de ontwikkeling van de koppelingskaart. De QTL-analyse onthulde een kwalitatief gen (*R39*) dat de fysiospecifieke resistentie verklaarde en drie QTLs (*RBQ1*, *RBQ2* en *RBQ3*) die de kwantitatieve resistentie verklaarden. Het kwalitatieve gen *R39* is een dominant gen dat een bijna volledige resistentie geeft tegen fysio NL16 in *L. saligna* CGN 5271 en daarom vertoont het vergelijkbare eigenschappen met *Dm*-genen (dominant fysiospecifieke resistentiegenen die een volledige resistentie geven tegen valse meeldauw; Engels: Downy mildew). De drie QTLs verklaarden 51 procent van de kwantitatieve

resistentie tegen fysio NL14, wat aangaf dat waarschijnlijk niet alle QTLs zijn waargenomen in deze F_2 populatie.
Naast deze nogal klassieke benadering van het karteren van resistentie met behulp van een F_2, hebben we een alternatieve strategie uitgevoerd gebaseerd op de ontwikkeling en beschrijving van een set geavanceerde terugkruisingslijnen, Backcross Inbred Lines (BILs). BILs zijn genetische gezien bijna identiek aan *L. sativa* maar bevatten één enkele introgressie (chromosoom fragment) van *L. saligna* CGN 5271 (Hoofdstuk4). Beginnend vanaf een F_1 plant, zijn BILs ontwikkeld door vier à vijf generaties terugkruisingen en één laatste generatie van zelfbestuiving. Alle terugkruisingen van de F_1 tot de BC_4 zijn willekeurig zonder bewuste selectie gemaakt. Merkergestuurde selectie is gebruikt vanaf de BC_4 generatie. Uiteindelijk is een set van 29 lijnen verkregen, die 95% van het genoom van *L. saligna* bedekt. Deze 29 lijnen behelzen 16 lijnen met één enkele homozygote introgressie (BILs), een lijn met twee homozygote introgressies, vijf lijnen met één heterozygote introgressie en zeven lijnen met twee of meer heterozygote introgressies. Verscheidene chromosoom gebieden vertoonden een erg scheve uitsplitsing in de F_2 populatie. Gebaseerd op uitsplitsingsverhoudingen in terugkruisingslijnen, konden we de scheve uitsplitsingen van drie chromosoom gebieden, waargenomen in de F_2, verklaren door loci die betrokken zijn bij pollen- of eicel fitness (levensvatbaarheid).
Toen het zaad van de eerste ontwikkelde BILs beschikbaar was, is er een ziektetoets opgezet om te verifiëren of de BILs, die een QTL bevatten volgens identificatie in de F_2-populatie, inderdaad een verhoogd niveau van kwantitatieve resistentie lieten zien. Negen BILs (of bijna BILs) zijn getest voor resistentie tegen *Bremia* fysio NL16. Samen omvatten ze 31% van het *L. saligna*-genoom. Twee loci voor resistentie, ontdekt in de F_2 (*R39* en *RBQ3*) zijn bevestigd in de ziektetoets met de BILs. *R39* is een dominant gen, dat volledige resistentie geeft tegen *Bremia* fysio NL16. *RBQ3* vermindert de aantastinggraad van de vatbare *L. sativa* met 49%, tien dagen na inoculatie. De kwantitatieve effecten van de resistentiegenen in deze BILs waren hoger dan verwacht volgens de F_2 resultaten. Voor *RBQ2* konden geen bepalende vergelijkingen gemaakt worden, omdat de introgressie in de terugkruisingslijn niet homozygoot aanwezig was. *RBQ1* is niet getest. Het meest enerverend was dat de BIL methode een nieuw resistentie locus onthulde op Chromosoom 8 met een reductie van de aantastinggraad van 77% vergeleken met de vatbare controle (tien dagen na inoculatie). Wij concluderen dat de BIL-karteringsmethode nieuwe QTLs kan onthullen die onopgemerkt blijven bij karteren in een F_2 populatie en deze methode maakt een kwantificatie van het effect van een resistentiegen in een *L. sativa*-achtergrond mogelijk.
Om onze kennis over niet-waard resistentie tegen *Bremia* in *L. saligna* uit te breiden, hebben we de genetica van niet-waard resistentie in *L. saligna* CGN 5271 vergeleken met die van een andere accessie, *L. saligna* CGN 11341. Gebaseerd op resultaten van AFLP-analyses, zijn de twee accessies voor 39% polymorf. We hebben de niet-waard resistentie van *L. saligna* CGN 11341 onderzocht door ziektetoetsen en DNA-merker analyses op een F_2- en een BC_1-populatie uit te voeren. Ziektetoetsen met *Bremia* fysio's NL14 en NL16 vertoonden een brede reikwijdte aan aantastinggradaties van

resistent tot vatbaar voor beide fysio's. De meerderheid van de planten had een vergelijkbaar resistentie niveau voor beide *Bremia* fysio's. Deze bevindingen impliceren dat de resistentie van *L. saligna* kwantitatief tot expressie komt en waarschijnlijk niet fysiospecifiek is. Enkele F_2 en BC_1 planten waren volledig resistent tegen *Bremia* fysio NL16 en tamelijk vatbaar voor fysio NL14. Een QTL analyse onthulde een hoofdgen voor resistentie op Chromosoom 9, die deze fysiospecifieke resistentie verklaart. Dit gen is *R39b* genoemd, omdat het verschillend zou kunnen zijn van *R39*.

Er zijn geen andere QTLs meer gedetecteerd in deze kleine F_2 populatie (n=54). Echter F_2 planten met *L. saligna* CGN 11341-allelen op loci van *RBQ1*, *RBQ2*, *RBQ3* en *RBQ4* die gekarteerd zijn in *L. saligna* CGN 5271, waren meer resistent dan F_2 planten met *L. sativa* allelen op deze loci. Wij concluderen dat het waarschijnlijk is dat dezelfde genen in beide *L. saligna's* de resistentie tegen *Bremia* verklaren. Een terugkruisingsprogramma voor een set BILs, die *R39b* en de loci voor QTLs uit *L. saligna* CGN 5271 omvat, vordert.

In het laatste hoofstuk van dit proefschrift worden enkele onderzoeksresultaten extra toegelicht. Wij beweren dat niet-waard resistentie in *L. saligna* niet verklaard wordt door een opeenstapeling van fysiospecifieke hoofdgenen voor resistentie (*Dm*-genen) maar door een resistentiemechanisme gebaseerd op QTLs. Verder maken we een vergelijking in efficiëntie tussen vier veredelingsmethoden om resistentiegenen uit *L. saligna* in te kruisen. Hieruit concluderen wij dat waarschijnlijk twee keer zoveel resistentiegenen ingekruist kunnen worden wanneer men merkergestuurde selectie toepast. Tenslotte worden verscheidene aanbevelingen gedaan aangaande onderzoek naar niet-waard resistentie en de wetenschappelijke toepassingen van BILs.

NAWOORD

Na een biologiestudie in het "wereldse" Utrecht kwam ik terecht als SLAIO bij de leerstoelgroep Plantenveredeling in de "stad" Wageningen. Nadat ik mij alle veredelaars-termen eigen had gemaakt, naast de biologie-termen die ik gewend was, begon ik aan een uitdagend onderzoek aan een nieuw gewas binnen de onderzoeksgroep, namelijk: sla. Het SLAIO-onderzoek verliep succesvol en ik deed het met veel plezier. Het eindresultaat is dit proefschrift. Een aantal mensen hebben een stempel gedrukt op dit sla-onderzoek en die wil ik hiervoor graag bedanken. Vele mensen hebben bijgedragen aan het plezier dat ik in mijn werk had en enkelen daarvan wil ik met naam noemen.

Allereerst wil ik Kees Reinink en Pim Lindhout bedanken, die tijdens hun reis naar Ales Lebeda in Tsjechië op het idee kwamen om een SLAIO-project te starten.

Dan wil ik mijn begeleider en copromotor, Pim Lindhout, bedanken. Ondanks zijn vele bezigheden kon ik altijd bij hem binnenlopen en wist hij tijd vrij te maken. Hij heeft me veel geleerd over karteren en resistentieveredeling en vooral ook over sociale en praktische zaken, zoals het onderhouden van goede contacten met bedrijven.

Daarnaast wil ik Piet Stam bedanken, mijn promotor. Bij Piet kon ik altijd terecht als ik het naadje van de kous wilde weten over de theorie en de software achter het maken van een koppelingskaart of het karteren van QTLs. Het verbaasde me vaak dat hij op een zo duidelijke en heldere manier, zulke ingewikkelde dingen kon uitleggen.

Rients Niks, mijn derde begeleider, wil ik bedanken voor zijn altijd aanwezige enthousiasme en interesse voor mijn "non-host" resistentieonderzoek. Ik vond het leuk en waardevol om met hem over "non-hosts" te discussieren. Verder wil ik hem, als taalpurist, bedanken voor al zijn taalkundige adviezen.

Fien Meijer-Dekens en Petra van de Berg wil ik bijzonder bedanken voor de technisch tips aangaande de AFLP techniek en het werkzaam maken van ons momela-lab. Petra wil ik nog speciaal bedanken voor de AFLP analyses op de BILs aan het eind van het project.

Alle medewerkers van Unifarm wil ik bedanken voor het verzorgen van mijn sla. In het bijzonder wil ik bedanken Jan Rijksen voor de organisatorische klussen, Pieter Kuiken, Bert Essenstam en Michel Hagendoorn voor de intensieve dagelijkse verzorging van mijn slaplanten en Anton Vels voor het urenlang bladponsen voor de ziektetoetsen.

Naast de veredelingsbedrijven ENZA zaden, Nickerson-Zwaan, Nunhems Zaden, Rijk Zwaan, Seminis en Syngenta, die het onderzoek financieel steunden, wil ik ook hun veredelaars en moleculaire biologen bedanken voor hun positieve bijdrage aan het onderzoek en de gezellige halfjaarlijkse bijeenkomsten. Helma Strookman en Loes Terlouw van Plantum (voorheen NVZP) waren ook altijd aanwezig bij deze bijeenkomsten en hen wil ik bedanken voor hun goede coördinatie van de samenwerking tussen de verdelingsbedrijven en ons (Wageningen Universiteit).

Verder wil ik Rik van Wijk en Johan Peleman van Keygene BV bedanken voor hun prettige samenwerking op AFLP gebied, wat een bijdrage heeft geleverd aan de publicatie van een sla-kaart (Hoofdstuk 2).

Furthermore, I want to thank Richard Michelmore, the lettuce professor from UC Davis, for his hospitality in his lab for two months.

Mijn twee Wageningse "sla"-collega's Clemens van de Wiel en Wim Koopman wil ik bedanken voor hun interesse en samenwerking.

Naast al het wetenschappelijk werk was er ook vaak tijd voor gezelligheid in de vorm van uitjes met onze personeelsvereniging, aio-etentjes of gewoon kletsen met collega's op de werkvloer en vooral in mijn werkkamer in de Terp en in het hoofdgebouw aan de Lawickse Allee. Ik wil al mijn kamergenoten bedanken voor hun gezelligheid en voor de interessante en soms heftige discussies: Taeho, Sylvan, Haider, Marjon, Guusje, Nelleke, Obembe, Sylvestre en Asun. Een kamergenoot, die ik speciaal wil bedanken voor de uitgebreide variété-voorstellingen van musical tot ballet en voor zijn computerprobleemoplossend vermogen is Hans van Os. Zonder Hans zou het een stuk saaier geweest zijn.
Tenslotte wil ik mijn vrienden en familie bedanken voor hun nimmer aflatende interesse voor sla. Ik hoop dat jullie door mijn uitleg over mijn sla-onderzoek begrepen hebben dat je planten ook kan verbeteren zonder genetische manipulatie.
Als laatste, maar niet het minste, wil ik ons pap, ons mam, ons Gemma en Peter en onze August bedanken voor de altijd aanwezige en onvoorwaardelijke steun.

CURRICULUM VITAE

Marieke Jeuken werd geboren op 29 februari 1972 te Eindhoven. In 1990 behaalde zij het Gymnasium diploma aan het St. Willibrordus Gymnasium te Deurne. In datzelfde jaar begon ze de studie Biologie aan de Universiteit van Utrecht. Tijdens de specialisatiefase deed zij drie afstudeervakken waarvan twee binnen Utrecht. Het eerste afstudeervak was bij de vakgroep Moleculaire Genetica en het tweede bij Biologische Toxicologie. Het derde afstudeervak vond plaats aan UC Berkeley in Amerika en was gerelateerd aan Plantenfysiologie. In september 1996 studeerde ze af met als hoofdrichtingen Moleculaire Plantkunde en Genetica.
In maart 1997 startte ze haar promotieonderzoek bij de leerstoelgroep Plantenveredeling. Sedert Juni 2001 is ze als postdoc aan deze groep verbonden.

The research described in this thesis was financed by the Dutch Lettuce Breeding companies and the Ministry of Economic Affairs (Senter).

Printed by Universal Press, Veenendaal