# Future perspectives of Backcross Inbred Lines for exploitation of wild germplasm: a case study on *Lactuca saligna* as a donor for quantitative resistance to lettuce downy mildew

**Marieke JW Jeuken and Pim Lindhout**
*Laboratory of Plant Breeding, Graduate School for Experimental Plant Sciences, Wageningen University, PO Box 386, 6700 AJ Wageningen, The Netherlands.*
*Contact: marieke.jeuken@wur.nl, Tel +31(0)317.483460*

**Abstract:** *Lactuca sativa* (lettuce) is susceptible to *Bremia lactucae* (downy mildew). *Lactuca saligna* (wild lettuce) is resistant to all downy mildew races and can be considered as a non-host. In this study the resistance of *L. saligna* was analyzed by two breeding strategies: (A) $F_2$ mapping strategy and (B) resistance mapping on Backcross Inbred Lines (BIL). The $F_2$ strategy revealed one *Dm* like gene (*R39*) and three QTLs. So far the BIL strategy revealed *R39* and five loci for quantitative resistance. Advantages and disadvantages of both strategies are compared and discussed.

**Keywords:** *Lactuca saligna*, downy mildew, nonhost resistance, QTLs, Backcross Inbred Lines, breeding strategy

## Introduction

Several *Lactuca* species are host for the biotrophic oomycete *Bremia lactucae* Regel (downy mildew). Because of major yield losses in lettuce (*Lactuca sativa* L.) 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. 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 over fifteen *Dm* genes have been identified and have been introgressed into commercial cultivars from cultivated germplasm sources or closely related species like *L. serriola* (Crute 1992, 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). 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 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 three *Lactuca* species (*L. serriola*, *L. virosa* and *L. saligna*) suggested that *L. saligna* is the only *Lactuca* species that can be crossed with cultivated lettuce (52 accessions tested) and is completely resistant to all *Bremia* races (20 races tested). Therefore, it may be considered a non-host (Bonnier et al.1992). 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 and possibly more durable as the known *Dm* genes that are all associated with necrosis (Hypersensitive Response). Very little is known about the genetics of resistance in non-host species (Heath 2001). It remains unclear whether the phenomenon "non-host resistance" comprises one or several defense

mechanisms explained by known or 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 the present study we investigated the genetics and specificity of *Bremia* resistance in *L. saligna.*

To this end two different strategies were used. One was a classical $F_2$ mapping strategy and the other strategy was using Backcross Inbred Lines (BILs). We choose to include this extra alternative strategy with the idea that in the case of complete downy mildew resistance in *L. saligna* classical $F_2$ approaches have not been successful in past breeding programs.

For the classical $F_2$ approach, an $F_2$ population was derived from a crossing between the resistant *L. saligna* CGN 5271 and susceptible *L. sativa* cultivar "Olof". All 126 $F_2$ plants were genotyped with molecular markers and phenotyped by *Bremia* disease tests (Fig. 1). In a QTL mapping procedure genotypic and phenotypic data were combined, to identify genomic regions that are involved in resistance.

In the alternative strategy resistance genes were mapped in a set of Backcross Inbred Lines (BIL). A set of BILs with introgressed genome regions of wild ancestors forms a genetic exotic library that can be screened infinitely. For tomato breeding, exotic genetic libraries have already proven to be useful in the transfer of agriculturally valuable traits of wild species (Zamir 2001). A set of lettuce BILs was developed by repeated backcrossing and Marker Assisted Selection based on the same original crossing between *L. saligna* CGN 5271 and *L. sativa* "Olof" as the one used for the $F_2$ population (Fig. 1); potentially each BIL harbors a single *L. saligna* introgression fragment in a *L. sativa* background, while all BILs together cover the total *L. saligna* genome.

In this manuscript the detection of resistance genes by the two strategies will be discussed and compared. The efficiency and practical usage will be considered.

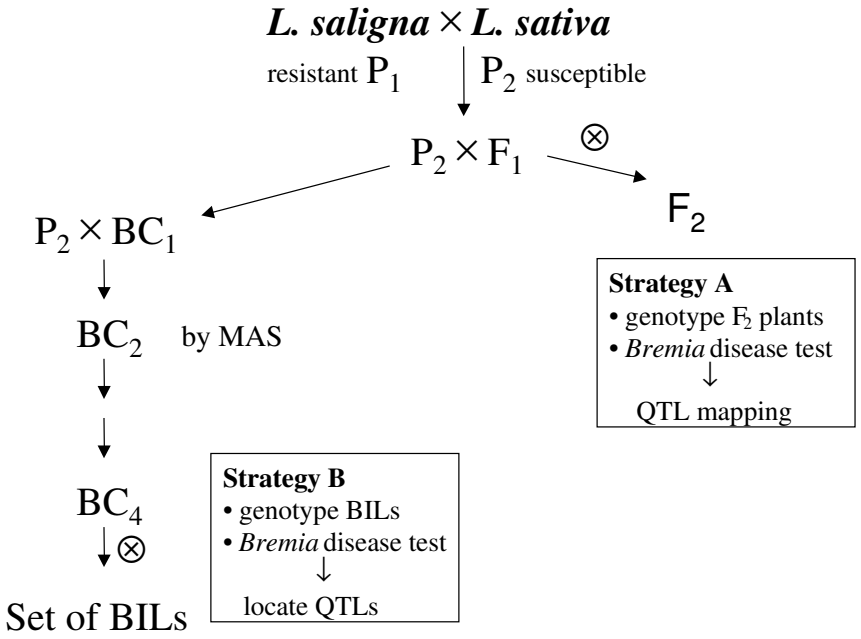


Figure 1. Working plan for the genetical dissection of the resistance of *L. saligna* to downy mildew by two strategies.
⊗ = selfing, MAS= Marker Assisted Selection

## Results

### *$F_2$ population*

All 126 $F_2$ plants were genotyped by AFLP analyses. An integrated interspecific AFLP map was constructed (Jeuken et al 2001).

All $F_2$ plants were 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 (Fig.1 in Jeuken and Lindhout 2002). QTL mapping revealed a gene (*R39*) with a qualitative effect involved in the race-specific resistance and three QTLs (*RBQ1*, *RBQ2* and *RBQ3*) involved in the quantitative resistance (Jeuken and Lindhout 2002).

### *Set of BILs*

Backcross Inbred Lines (BILs) were developed in which chromosome segments of *L. saligna* were introgressed into *L. sativa* (Fig. 1). These lines were developed by four to five backcrosses and one generation of selfing. The first three generations of backcrossing were not selected. 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) and four lines had two homozygous introgressions (double-BILs). The other nine lines are still segregating for one to three introgressions from *L. saligna*. These lines are yet in the process of BIL development. Together, the 20 BILs covered about 70% of the *L. saligna* genome. By BIL association mapping several morphological traits viz. 'pointed leaf apex', 'reflexed involucre', 'early bolting' and 'long dark-green leaves' and several additional AFLP markers were mapped.

In a preliminary disease test on leaf discs (similar to the tests on the $F_2$ population), we exposed six BILs to downy mildew race NL16 to test whether the BILs, which carried QTLs as identified in the $F_2$ population, showed enhanced levels of quantitative resistance indeed (data not shown). The disease test on the BILs confirmed two resistance loci detected in the $F_2$ population (*R39* and *RBQ3*). *R39* gave a complete resistance against *Bremia* race NL16. *RBQ3* reduced 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. *RBQ1* and *RBQ2* could not been tested due to the absence of a line with one of these QTLs in a homozygous state. Most exciting, the BIL method revealed a new resistance locus, designated *RBQ4*, on Chromosome 8 with a 77% reduction on the infection severity compared to the susceptible control ten days post inoculation.

Recently, three disease tests with *Bremia* races NL14 and NL16 were performed on twenty BILs that covered about 70% of the *L. saligna* genome. From these BILs fifteen showed an infection severity similar to the susceptible parent *L. sativa "Olof"* (data not shown). This is in agreement with the fact that no resistance genes have been mapped before on the *L. saligna* introgression segments that were covered by these fifteen BILs.

From the twenty BILs, two BILs confirmed previously detected resistances. BIL 9.2 with *RBQ3,* detected in the $F_2$, showed a reduction in the infection severity of 50% and 72% to NL16 and NL14 compared to the susceptible parent (Table 1). *RBQ4* on Chromosome 8 (BIL 8.2), not detected in the $F_2$, indicated again resistance but with a slightly less dramatic reduction in infection severity ranging from 23 to 62% compared to the preliminary disease test on BILs (Table 1).

Most exciting was the indication of three new BILs with resistance (BIL A, B and C). The resistance genes from these three BILs have not been detected before in the $F_2$

population. The levels of resistance are quite high with a reduction of the infection severity ranging from 61% to 55% for BIL A to BIL C.

*RBQ1* and *RBQ2* could not been tested due to the absence of a line with one of these QTLs in a homozygous state. For the development of BILs for *RBQ1* and *RBQ2* already 30 and 40 plants from a line with a single introgression have been AFLP fingerprinted. However, in these selfing lines that are segregating only for the *L. saligna* introgression with one of the QTLs, not a single plant has been detected with the *RBQ1* or *RBQ2* locus in a homozygous state.

Table 1. Twenty Backcross Inbred Lines (BILs) were tested for resistance to *Bremia* race NL16 and NL14 (two independent tests). Only the results of *L. sativa* Olof, *L. saligna* CGN 5271 and BILs that indicated resistance are presented in this table. The average infection severity scores per line are presented as percentage of leaf area covered with sporangiophores of *Bremia* 10 days after inoculation. Per BIL minimally seven plants were tested. Per plant four leaf discs were tested.

| BILs/lines | Average infection severity (in %)$^{\delta}$ | | |
|---|---|---|---|
| | NL16 | NL14 | NL14 |
| BIL 9.2 | $44^{bc}$ | $31^{b}$ | $25^{b}$ |
| BIL A | $36^{b}$ | $35^{b}$ | $34^{b}$ |
| BIL B | $52^{bcd}$ | $44^{bc}$ | $56^{b}$ |
| BIL C | $63^{bcd}$ | $54^{bcd}$ | $39^{b}$ |
| BIL 8.2 | $67^{bcd}$ | $60^{bcd}$ | $33^{b}$ |
| *L. sativa* Olof | $87^{d}$ | $89^{d}$ | $87^{c}$ |
| *L. saligna* CGN5271 | $0^{a}$ | $0^{a}$ | $0^{a}$ |

$^{\delta}$Letters in common within a column, indicate that the values are not significantly different ($\alpha$ =0.05, Tukey HSD procedure)

## Discussion

A sum of the results of both strategies shows that by using the $F_2$ strategy *R39*, *RBQ1*, *RBQ2* and *RBQ3* were detected and that by using the BIL strategy *R39*, *RBQ3* and four new QTLs were identified, but no conclusions can be made yet about the confirmation of *RBQ1* and *RBQ2*. When the strategies are compared in detecting resistance genes we can conclude that so far the BIL strategy was more efficient as by using this approach four new QTLs were identified.

Besides differences in detection of resistance genes, each strategy has its own qualities and the sum of all advantageous and disadvantageous properties determines which strategy a breeder will choose. In the following lines we will sum up the advantages and disadvantages of both strategies in this case study on nonhost resistance in *L. saligna* to downy mildew in order to make a proper deliberation about the efficiency of the strategies.

***$F_2$ strategy***

Advantages

- The development of an $F_2$ population only takes two generations starting from the original cross between the recurrent parent and the wild species parent. This procedure is relatively fast compared to the development of BILs. This allows rapid screening of plant material for resistance.

- When a trait is expressed as a result of a genetic interaction with a positive effect, the trait is still detectable in certain genotypes in the $F_2$ population but the genetic analysis will be rather difficult.

Disadvantages

- $F_2$ populations of plant species from crosses of cultivated species with wild ancestors are often limited in size and plants may have weak vigor and fertility. Moreover, replications are impossible, which altogether negatively influences the quality of the screening experiment.
- Genetic and morphological dissimilarity between $F_2$ plants from wide crosses influence the measurements of experiments, which gives a less precise quantitative assessment of the trait.
- $F_2$ populations of wide crosses often show distorted segregations that result in underrepresentation of certain genotypes. In this way genes may go unnoticed or their effects may be underestimated.

***BIL strategy***

Advantages

- BILs are immortal lines that allow large scale experiments in many replicates and in many conditions/environments. This enhances the quality of the experiment and also allows genotype×environment interactions to be studied.
- The high genetic and morphological similarity between BILs and the recurrent parent enables precise estimates of traits. When the recurrent parent species is a cultivated crop species, the effect of the trait can be directly extrapolated to the commercial cultivars. Furthermore, the genes responsible for the trait can be introgressed rapidly into commercial cultivars as compared to introgression starting from $F_2$ plants.
- Genes that go unnoticed in the $F_2$ population may be detected in BILs. Several mechanisms may cause this effect. First, the homogeneous genetic background of BILs, as compared to an $F_2$, will enhance the detection power for single genes. Second, and related to this, certain genotypes may be under- or overrepresented in an $F_2$, so that their effect is easily masked by other genes. Third, epistatic interactions between unlinked genes may mask the 'main effect' of singles genes in an $F_2$, whereas in a BIL such interactions are absent.

Disadvantages

- The development of a set of BILs that covers the complete genome of the wild species takes about six generations starting from the original cross between the recurrent parent and the wild species parent ($F_1$, $BC_1$, $BC_2$, $BC_3$, $BC_4$ and $BC_4S_1$). Compared to an $F_2$ population this takes a long time before the test material is ready for use.
- The development of a set of BILs is very labor-intensive and costly as many crosses have to be made and many plants have to be fingerprinted with markers.
- When a trait is expressed as a result of genetic interactions from not-closely-linked genes, the trait is not detectable anymore in BILs.

Overall it is noticed that many factors play a role in the choice for one of the breeding strategies. Some factors are easy to predict, like time for the generation of test material ($F_2$ plants $\leftrightarrow$ BILs) or costs of Marker Assisted Selection. In contrast, the occurrence of certain other factors like sterility or distorted segregation may be expected but the magnitude of their

effect is difficult to predict. A factor, which is virtually unknown in advance, is the occurrence of epistatic interactions between (linked or unlinked) genes that control the trait.

In this case study on nonhost resistance in *L. saligna* the BIL strategy has yielded already two more QTLs than the $F_2$ strategy (four new QTLs form the BIL approach and two QTLs detected in the $F_2$ are under investigation in the BIL approach) while downy mildew resistance has still to be tested in nine future BILs. Therefore, the BIL strategy is more efficient. Furthermore, the BIL strategy tends to reveal QTLs that are not involved in genetic interactions; this makes the introgression of the trait in commercial cultivars simpler. As the BIL strategy itself resembles the introgression of a trait into a commercial cultivar, it already reveals pitfalls for introgression. An example of a pitfall is the observed strong prevalence of recipient alleles in the regions that harbor *RBQ1* and *RBQ2*, which so far prevented the recovery of homozygotes for these regions.

In conclusion, we state that if a breeding company is prepared to invest time and money in the development of a set of BILs, this seems the most efficient strategy for the introgression of quantitative resistance. The $F_2$ strategy will be efficient if a single dominant gene controls the trait, or one expects the trait to be expressed through epistatic interactions of a few genes.

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## References

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

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

Heath MC, 2001. Non-host resistance to plant pathogens: nonspecific defense or the result of specific recognition events? Physiol Mol Plant Pathol 58:53-54

Jeuken M and Lindhout P, 2002. *Lactuca saligna*, a non-host for lettuce downy mildew (*Bremia lactucae*) harbors a new race specific *Dm* gene and three QTLs for resistance. Theor Appl Genet 105:384-391

Jeuken M, van Wijk R, Peleman P and Lindhout P, 2001. An integrated interspecific AFLP map of lettuce (*Lactuca*) based on two *L. sativa* × *L. saligna* $F_2$ populations. Theor Appl Genet 103:638-647

Lebeda A and Reinink K, 1994. Histological characterisation of resistance in *Lactuca saligna* to lettuce downy mildew (*Bremia lactucae*) Physiol Mol Plant Pathol 44:125-139

Reinink K, 1999. Lettuce resistance breeding. In: Lebeda A and Křístková E (eds) Proc. EUCARPIA Meeting on Leafy Vegetables, Olomouc Czech Republic pp 139-147

Van Ettekoven K and 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

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