# Progress towards mapping QTLs for pest and disease resistance in lettuce

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**Abstract**: Quantitative non race-specific resistance may be more durable than resistance based on single dominant genes. However selection for multigenic traits in a breeding programme is difficult. We have produced a lettuce mapping population of recombinant inbred lines using cv. Iceberg, a source of quantitative resistance to downy mildew and peach-potato aphid, and have tested the lines for resistance. We are constructing a genetic linkage map from the population using molecular markers which we will use to identify QTL for the resistances.

**Keywords:** *Lactuca sativa*, quantitative resistance, *Bremia lactucae*, *Myzus persicae*, genetic map, marker assisted selection.

## Introduction

Retailers and consumers demand high quality 'blemish-free' fresh produce but there is increasing consumer concern and legislation over pesticide use and residues in food crops. Host plant resistance to pests and diseases is therefore a major target in most plant breeding programmes. Historically breeders have chosen to utilise resistances determined by single dominant genes since these are easier to handle in a breeding programme. This type of resistance has inevitably broken down since there is often a gene-for-gene host/pathogen relationship (Johnson et al., 1977) and the growing of a resistant variety selects for the matching virulence in the pathogen population. This leads to a 'boom and bust' cycle of varietal production. There are alternative forms of resistance which are apparently non-race specific and likely to be more durable. However this resistance is often quantitative, may be multigenic and is subject to environmental influence (Crute and Norwood, 1981). It has not been as widely used in plant breeding because of the difficulty of selecting plants posessing all the genes for resistance and having good quality characteristics. The availability of molecular markers now offers the potential for marker assisted selection (MAS) of multigenic traits.

The objective of our studies is to develop genetic resources and techniques for MAS of quantitatively inherited resistance to a major pest and disease in lettuce, the peach-potato aphid (*Myzus persicae*) and lettuce downy mildew (*Bremia lactucae*).

Foliar aphids are major insect pests of field lettuce (Reinink and Dieleman, 1993). Varieties with resistance to some aphid species are available e.g. 'Dynamite' (van der Arend et al., 1999), but there are no current varieties with resistance to the peach-potato aphid. Growers therefore still need to use insecticide even if they are growing varieties with resistance to other lettuce infesting aphid species. Lettuce downy mildew is the most important lettuce disease worldwide. Resistant lettuce varieties have been produced by breeding using single dominant (Dm) genes, but resistance generally breaks down within a few years (Lebeda and Zinkernagel, 1999). We have developed a population of recombinant

inbred lines (RILs) for genetic mapping based upon the cross between the lettuce cultivars 'Saladin' and 'Iceberg'. Iceberg (syn. Batavia Blonde a Bord Rouge) is an old variety which has been grown since the mid-1800's and is a source of quantitative resistance to both downy mildew (Table 1) and peach-potato aphid (Reinink *et al*., 1988). We have tested RILs for resistance to downy mildew and peach potato aphid and we are using DNA from these lines to construct a genetic linkage map with molecular markers. Our objective is to combine the phenotype and genotype data to identify QTLs for resistance.

Table 1. Comparison of field resistance to downy mildew in lettuce cultivars.

| Cultivar | % Infection | No. of leaves infected (± S.E.) |
|---|---|---|
| Unrivalled (control) | 86 ± 5 | 2.4 ± 0.3 |
| Iceberg | 23 ± 6 | 0.2 ± 0.1 |
| Grand Rapids | 23 ± 9 | 0.3 ± 0.1 |
| Batavian Blonde de Paris | 36 ± 13 | 0.6 ± 0.3 |

## Materials and Methods

***Plant material***

From an initial cross of *L. sativa* cv. 'Saladin' x cv. 'Iceberg' an $F_2$ population of 500 plants was produced to initiate a single seed descent (SSD) programme. The resultant population of 340 $F_6$ recombinant inbred lines (RIL) was used for phenotypic assessment of aphid and downy mildew resistance. For genetic linkage map construction we used two mapping populations: ca.200 of the $F_5$ parental plants of the $F_6$ RILs and an additional 118 $F_2$ individuals.

***Tests for resistance to B. lactucae***

Replicated field trials to assess downy mildew resistance took place at five sites in the UK, USA and Netherlands. Fifty RILs were tested at all sites, to obtain estimates of between-site environmental variation. An additional 48 RILs per site were tested to maximise the number of lines assessed. Each trial was arranged in two replicate blocks using an alpha design. At one site, natural infestation was allowed to take place. At all other sites spreader beds were inoculated with an appropriate isolate of *B. lactucae* selected to overcome any major gene resistance in the RILS (inherited from Saladin). Six plants of each line from each block were scored on a scale of 1 to 5, score 1 being free from disease or one or two tiny lesions through to 5 indicating heavy sporulation in joined up lesions. REML analysis was used to determine any differences in resistance between lines.

***Tests for resistance to M. persicae***

For *M. persicae* resistance 240 of the RILs tested for downy mildew resistance, were also tested in controlled environment chambers against a single aphid clone. Each line was tested 8 times. Six adult wingless *M. persicae* that had only recently started reproducing were taken from culture plants (*Brassica oleracea* var. *gemmifera* cv. Oliver) and were placed on individual lettuce plants. The plants were then bagged to prevent aphids from moving between plants. After three days the number of nymphs produced on each plant was recorded and all adult aphids removed. After a further two days the survival of these nymphs was measured by counting the number of aphids that remained on each plant. This final number of aphids per plant was subjected to REML analysis to determine any differences in resistance between lines.

***Molecular marker analysis***

DNA was extracted from $F_5$ and $F_2$ plants and used for analysis with PCR-based molecular markers. RAPD analysis was carried out on the $F_2$ population only using random 10-mer oligonucleotide primers under standard conditions (Welsh and McClelland, 1990). Both the F2 and $F_5$ populations were analysed using AFLP according to the method of Vos et al. (1995). Polymorphic markers were scored as dominant (i.e. bands present or absent). Each gel was scored twice to minimise errors. Linkage analysis on both mapping populations was performed using JOINMAP 3.0 software (Van Ooijen and Voorrips, 2001). Markers were assigned to linkage groups based on pairwise recombination frequencies and LOD (logarithm of odds) values. LOD values were increased in steps of 0.5 units for assessment of the stability of groupings. Mapping of markers within linkage groups was carried out using the Kosambi mapping function, with a pairwise recombination upper limit of 0.45 and a LOD threshold of 0.01. The two maps were combined to produce an integrated map using using JOINMAP 3.0 based on linkage groups with common markers. QTL analysis was carried out by marker regression (Kearsey and Hyne, 1994).

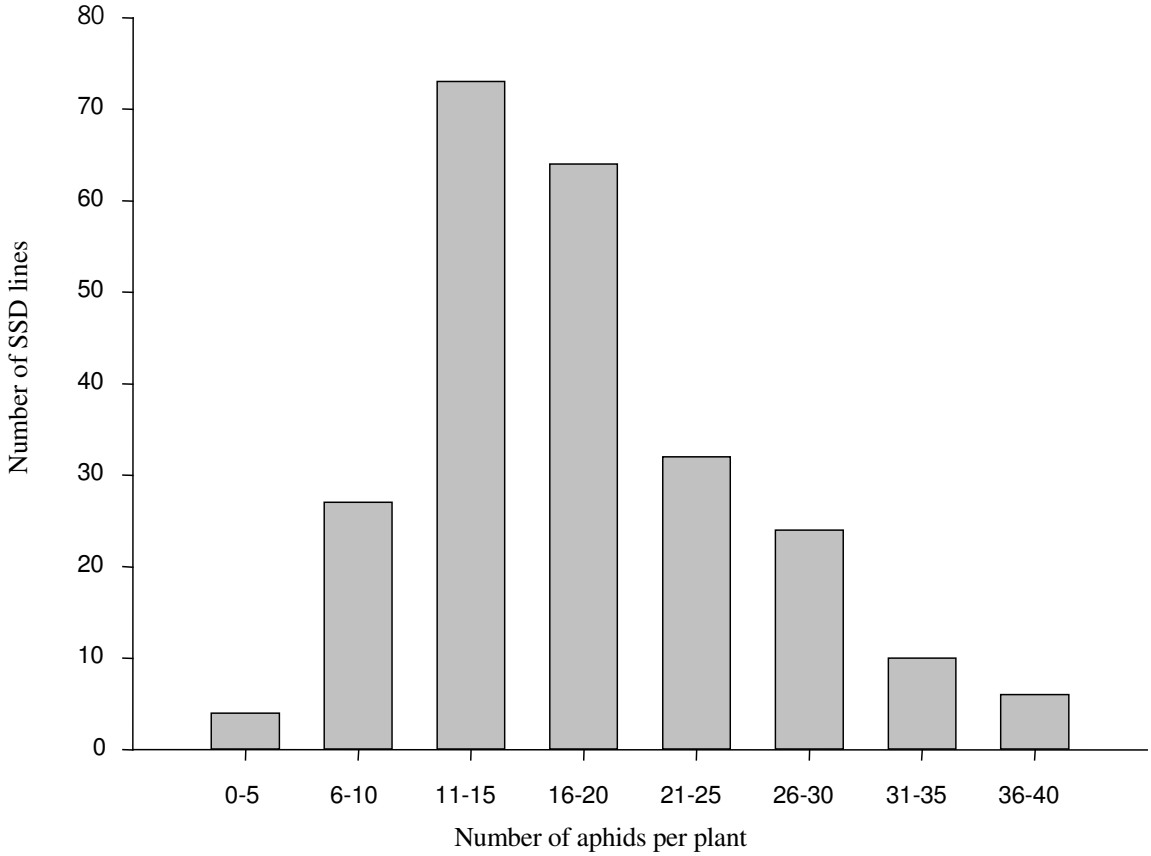


Figure 1. The distribution of the mean number of *M. persicae* per plant for each of 240 SSD $F_6$ lines created from the cross between Iceberg (resistant) and Saladin (susceptible).

## Results and Discussion

***M. persicae resistance***

There was continuous variation in the number of aphids supported on the RILs (Figure 1), with the distribution skewed towards resistance (i.e. fewer aphids). The most resistant lines supported significantly fewer aphids than the most susceptible.

Further work is underway to test selected resistant and susceptible lines with more aphid clones to determine whether this resistance is non-clone specific.

***B. lactucae resistance***

There were significant differences in disease scores between the lettuce RILs at each trial site, giving a range of scores as expected for this quantitative trait. Lines were found with resistance equivalent to Iceberg, and susceptibility equivalent to Saladin. The results for lines tested across all sites were largely consistent between sites. Table 2 shows the mean disease score and the range of scores across sites for the five most resistant and most susceptible lines.

Table 2. Mean downy mildew disease scores of the most resistant and most susceptible Saladin x Iceberg lettuce lines tested at five sites.

| Resistant | Range across sites | Susceptible | Range across sites |
|---|---|---|---|
| 2.54 | 1.09 - 4.00 | 3.99 | 3.77 - 5.00 |
| 2.75 | 1.02 - 4.00 | 3.99 | 3.09 - 5.00 |
| 2.80 | 1.16 - 4.25 | 3.95 | 3.55 - 5.00 |
| 2.82 | 1.11 - 4.50 | 3.89 | 2.76 - 5.00 |
| 2.90 | 1.11 - 4.00 | 3.82 | 2.45 - 4.75 |
| (Iceberg 1.85) | (1.00 - 2.75) | (Saladin 3.60) | (2.17 - 5.00) |

***Molecular marker analysis***

RAPD primers and AFLP primer pairs were screened for polymorphism between the mapping parents. To date, 14 RAPD primers and 17 AFLP primer pairs have been used to generate informative amplification products, giving more than 180 polymorphic loci for map construction. RAPD markers have been scored in the $F_2$ mapping population and AFLP markers have been scored in both populations. The $F_2$ data produced 18 linkage groups covering approx. 600 cM of map length, the $F_5$ data produced 11 linkage groups covering approx. 450 cM. A combined map was constructed containing 14 linkage groups covering 610 cM. One linkage group showed segregation distortion over its whole length. Similar distortion was reported by Jeuken et al. (2001). Further work is under way using additional markers to increase the map coverage and to 'anchor' the map to other published lettuce genetic maps.

Initial QTL analysis carried out using the phenotype and map data available has identified putative QTLs for resistance to aphids and downy mildew on several genetic linkage groups. We are working to locate these QTLs more precisely. The identification of QTL for quantitative resistance will provide tools to lettuce breeders who wish to introduce this type of resistance instead of or in combination with single gene resistance.

## Conclusions

We have developed $F_6$ lettuce RILs which segregate for quantitative resistance to *B. lactucae* and *M. persicae*, as shown by replicated trials. We have also produced genetic linkage maps based upon the $F_5$ RILs and an $F_2$ population. Our initial analyses suggest several possible QTLs for resistance to both downy mildew and *Myzus persicae.* We are carrying out further work to refine the map and QTL positions.

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## References

Crute, I.R. and Norwood, J.M. 1981. The identification and characteristics of field resistance to lettuce downy mildew (*Bremia lactucae* Regel). Euphytica 30: 707-717.

Jeuken, M., van Wijk, R., Peleman, J. and Lindhout, P. 2001. An integrated interspecific map of lettuce (Lactucae) based on two *L. sativa* x *L. saligna* F2 populations. Theor. Appl. Genet. 103: 638-647.

Johnson, A.G., Crute, I.R. and Gordon, P.L. 1977. The genetics of race specific resistance in lettuce (*Lactuca sativa*) to downy mildew (*Bremia lactucae*). Ann. Appl. Biol. 86: 87-103.

Kearsey, M.J. and Hyne, V. 1994. Qtl Analysis - a Simple Marker-Regression Approach. Theor. Appl. Genet., 89: 698-702.

Lebeda, A. and Zinkernagel, V. 1999. Durability of race-specific resistance in lettuce against lettuce downy mildew (*Bremia lactucae*). *In:* Proc. EUCARPIA Meeting on Leafy Vegetables, Olomouc, Czech Republic, 8-11 June 1999. Pp. 183-189.

Reinink, K., Dieleman, F.L. and Groenwold, R. 1988. Selection of lines of lettuce (*Lactuca sativa* L) with a high-level of partial resistance to *Myzus persicae* (Sulzer). Euphytica 37: 241-245.

Reinink, K.and Dieleman, F.L. 1993. Survey of aphid species on lettuce. IOBC/WPRS Bulletin 16/5: 56-68.

Van der Arend, A.J.M., Ester, A. and van Schijndel, J.T. 1999. Developing an aphid resistant butterhead lettuce 'Dynamite'. *In:* Proc. EUCARPIA Meeting on Leafy Vegetables, Olomouc, Czech Republic, 8-11 June 1999. pp. 149-157.

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.

Van Ooijen, J.W. and Voorrips, RE. 2001. JoinMap® 3.0, Software for the calculation of genetic linkage maps. Plant Research International, Wageningen, the Netherlands.

Welsh, J. and McClelland, M. 1990. Fingerprinting genomes using PCR with arbritary primers. Nucleic Acids Res. 18: 7213-7218.