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SID 5  Research Project Final Report

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### Project identification

1. **Defra Project code**  
   HH3723SX

2. **Project title**  
   Crop Improvement of Field Vegetables

3. **Contractor organisation(s)**  
   Warwick HRI
   University of Warwick
   Wellesbourne
   Warwick
   CV35 9EF

4. **Total Defra project costs**  
   (agreed fixed price)  
   £ 3,653,148

5. **Project:**  
   start date..............  
   01 April 2004

   end date...............  
   31 March 2009
6. It is Defra’s intention to publish this form. Please confirm your agreement to do so.................................................................YES ☐ NO ☐

(a) When preparing SID 5s contractors should bear in mind that Defra intends that they be made public. They should be written in a clear and concise manner and represent a full account of the research project which someone not closely associated with the project can follow.

Defra recognises that in a small minority of cases there may be information, such as intellectual property or commercially confidential data, used in or generated by the research project, which should not be disclosed. In these cases, such information should be detailed in a separate annex (not to be published) so that the SID 5 can be placed in the public domain. Where it is impossible to complete the Final Report without including references to any sensitive or confidential data, the information should be included and section (b) completed. NB: only in exceptional circumstances will Defra expect contractors to give a "No" answer.

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(b) If you have answered NO, please explain why the Final report should not be released into public domain

Executive Summary

The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

The project has successfully carried out its purpose to develop a range of underpinning resources and information about genetic variability for specific traits of interest, in a form suitable for use in crop improvement research in vegetable brassicas, lettuce and onion. The project has had significant interaction with and technology transfer to industry as well as delivering plant material and information to other research projects. Overall the project has been instrumental in generating approximately £3m of additional funding for crop improvement research.

The project had two components

- Resource building activities
- Trait based research

Resources development activities in the project have resulted in the development of underpinning resources for genetic improvement research in brassica, onion and lettuce.

Brassica vegetables (B. oleracea) includes cauliflower, broccoli, cabbage, Brussels sprouts, kohlrabi and kales. However, genetic improvement of these crops is largely limited to breeding crosses within each crop. This limits the utilisation of genetic variation which exists in other crops that may be of benefit for sustainable crop production. There are over 4,400 accessions of the different forms of vegetable brassicas and related wild species maintained in the Genetic Resources Unit at Warwick HRI. The large size of the collection causes problems with regard to how users access the variation they represent as it is often not possible to assess all the accessions for a given trait. To overcome this difficulty, we have pioneered the development of diversity fixed foundation sets (DFFS). These are based on the concept of a diversity core collection in which a manageable number of lines are selected to represent the genetic diversity of the species with minimum duplication, but with the added benefit that genetically fixed, or true breeding, lines are generated from each member of the ‘core’ foundation set selected to provide a structured sample of variation within the genepool. Within this project we have been developing DFFS for the domesticated forms of B oleracea (cauliflower, broccoli, cabbage, Brussels sprouts, kohlrabi and kales) and a DFFS from wild species related to B oleracea. Diversity analysis has shown that the wild species are much more genetically diverse than the domesticated crops and given the resource poor ecological niches in which they exist we anticipate that the wild relatives of vegetable brassica crops are potential sources of beneficial alleles for traits important for sustainable production.

The project has also regenerated two reference brassica mapping populations which are key to brassica genetic research to ensure that they are publicly available; these are the ‘AG’ mapping population consisting of DH lines derived from the cross between the Chinese kale DH line A12 and the broccoli DH line GD33 derived from the F1 hybrid cv Green Duke and the AG substitution line (AGSL) population which is derived from the same original cross as the AGDH population and has significant value in providing the ability to take extend any genetic analysis carried out using the AGDH population.

For onion the strategy for producing an onion DFS was to attempt to minimise the intra accession variation and to characterise it while maintaining sufficient heterogeneity to maintain viability of the accessions. This has been done by producing half sib families (the seed within each half sib family is all derived from the same female parent but from a number of male parents) from 95 gene bank accessions of onion; these are now available for follow on research.
For lettuce the project has regenerated the Saladin x Iceberg mapping population of inbred lines and has improved the associated linkage map. These are key resources for trait based research in lettuce.

Trait based research has involved:

- **Resistance to black rot (Xanthomonas campestris pv campestris)** – A genetic analysis was carried out of the resistance in Chinese cabbage (B rapa) to the two most common races of this pathogen. It was not possible to transfer the resistance to B oleracea.

- **Resistance to cabbage aphid (Brevicoryne brassicae)** - Research initiated in HH0910SFV to produce breeding material with resistance to the cabbage aphid derived from 2 wild species B. cretica and B. incarna was completed and the material released to commercial brassica breeding companies.

- **Resistance to downy mildew of brassica (Hyaloperonospora parasitica subsp. Brassica)** - Three sources of resistance identified previously in an EU funded project were shown to be controlled by the same gene. The resistance has been transferred into a broccoli crop type. This material is now available for experiments aimed at integrating host resistance with other forms of disease control such as disease forecasting being developed at Warwick HRI. However, although the initial testing against 25 isolates indicated the resistance might be broad spectrum subsequent testing against other UK isolates showed that the resistance could be overcome. Screening of the wild species diversity set identified some potential sources of partial resistance.

- **Resistance to Turnip Mosaic Virus (TuMV)** - Significant progress has been made in mapping novel dominant broad-spectrum TuMV resistance in Brassica rapa. Further work is needed to map the genes and hence develop molecular markers and determine their potential for transfer to the B. oleracea genome by non-transgenic means. Other sources of resistance to TuMV have all been identified in B. rapa. Thirty five lines of wild Brassica spp diversity were tested for resistance to TuMV isolates All lines were susceptible to the pathotype 1 isolates which are the most common in the UK.

- **Genetic analysis of shelf life in broccoli** - The genetic analysis was completed by increasing the number of lines in the mapping population and assessing them for their post harvest shelf life. The data and the mapping population was released to a BBSRC funded Industrial partnership award (Mapping and analysis of genetic loci controlling quality traits in broccoli) involving Syngenta Seeds.

- **Leek rust (Puccinia porri)** resistance - Leek rust resistant breeding lines were released to the world market leader in commercial leek breeding.

- **Genetic analysis of nitrate content of lettuce** - Thirteen genetic factors for nitrate accumulation in lettuce have been identified; several of these co-locate on the genetic map, strongly suggesting a role in controlling nitrate level. However, we have no knowledge of the mechanism underlying the QTL or whether they are associated with nitrate metabolism, transport or accumulation. This limits their exploitation in a breeding programme. In future work we will initiate work to ‘dissect’ the complex trait of nitrate level to better inform breeding programmes aimed at improving the ‘nitrate economy’ of the lettuce crop.

- **Field Resistance to lettuce downy mildew (Bremia lactucae)** - An additional genetic factor for a defined component of field resistance to (latent period) was identified using a newly developed lab based test. The data from this project have been used in a parallel collaborative project with a commercial lettuce breeding company on developing a strategy for breeding for FR to B. lactucae. This work is ongoing and there is a need to fine map the genetic factors for resistance in order to identify tighter molecular markers. Ultimately it would be of great interest to identify the genes for FR as this would provide insight into the mechanism of this type of resistance and also possible reasons for its durability.

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**Project Report to Defra**

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:

- the scientific objectives as set out in the contract;
- the extent to which the objectives set out in the contract have been met;
- details of methods used and the results obtained, including statistical analysis (if appropriate);
- a discussion of the results and their reliability;
Objectives: The project originally had 18 objectives; these were divided across the crops worked on in the project as follows:

Vegetable brassicas
1. Produce a strategic resource of fixed lines of *B. oleracea* for use in research and crop improvement by developing a *B. oleracea* DFFS.
2. Regenerate key reference *B. oleracea* DH mapping populations.
3. ‘Capture’ genetic variation present in the secondary genepool of C genome wild species in a form that is utilisable as a strategic resource by breeders and researchers for crop improvement of vegetable brassicas by developing a C genome DFFS.
4. Transfer resistance to *Xanthomonas campestris pv campestris* from *B. rapa* to *B. oleracea*.
5. Release breeding lines with resistance to *Brevicoryne brassicae* to breeding companies.
6. Determine whether monogenic resistance to downy mildew derived from different sources are allelic or distinct from one another.
7. Introgress at least 2 sources of downy mildew resistance into a crop phenotype.
8. Identify new sources of downy mildew resistance in a foundation set of wild C-genome species, and capture the resistance by transferring it into a rapid cycling background.
9. Identify if pathogenicity factors are common between *Brassica* and Arabidopsis isolates of *Peronospora parasitica* (downy mildew) and assess if these can be used to screen for resistance in the C genome foundation set.
10. Identify loci for pathotype-specific and broad-spectrum resistance to TuMV in the *Brassica* A genome to determine whether any are amenable to transfer to the C genome.
11. Identify new sources of TuMV resistance derived from wild C-genome species.
12. Complete a QTL analysis of shelf life in broccoli.

Alliums
13. Release breeding lines with leek rust resistance to breeding companies.
15. Develop a methodology to identify novel sources of resistance to onion downy mildew.

Lettuce
16. Generate a generic resource for lettuce crop improvement
17. Complete a genetic analysis of nitrate content of lettuce.

The methods used and the results obtained are described below for each objective, together with discussion of the extent to which each objective has been met and possible future work.

1. Produce a strategic resource of fixed lines of *B. oleracea* for use in research and crop improvement by developing a *B. oleracea* DFFS.

Introduction: Brassicas are especially notable for their genetic diversity and individual species often include multiple crop types; for *B. oleracea* this includes cauliflower, broccoli, cabbage, Brussels sprouts, kohlrabi and kales. However, genetic improvement of these crops is largely limited to breeding crosses within each crop, leading to genetic erosion of the allelic diversity of the crop. This limits the utilisation of alleles which are likely to exist in other crops that would be of benefit for sustainable crop production. There are over 4,400 accessions of the different forms of vegetable brassicas and related wild (C genome) species maintained in the Genetic Resources Unit at Warwick HRI. The large size of the collection causes problems with regard to how users access the variation they represent as it is often not possible to assess all the accessions for a given trait. To overcome this difficulty, we have pioneered the development of diversity fixed foundation sets (DFFS). These are based on the concept of a diversity core collection (1) in which a manageable number of lines are selected to represent the genetic diversity of the species with minimum duplication, but with the added benefit that genetically fixed, or true breeding, lines are generated from each member of the ‘core’ foundation set selected to provide a structured sample of variation within the *B. oleracea* genepool. The 376 founder lines of, divided into 4 subsets of 94 lines, were identified and sourced for the BoiDFFS prior to the start of this project including the parents of the *B. oleracea* mapping populations available at Warwick HRI. This project focussed on producing genetically fixed lines from the first two subsets of founder lines. The BoiDFFS will therefore provide a unique publicly available strategic resource complementary to the *B. napus*-DFFS which is being established in the Defra-funded OREGIN project.

Methods and Results: DNA has been extracted from all parental founder plants and securely stored at −80°C and a sub-sample of each DNA sample has undergone whole genome amplification using Phi DNA polymerase. The ‘Genomiphied’ DNA is available for future projects and for public distribution. The diversity within the first ‘subset’ of 94 foundation lines was assessed using 100 mapped SSR markers. In total this identified 1006 alleles;
503 of these found in > or =5 accessions were used for diversity analysis (Fig 1). This showed the accessions grouping together by crop type. However, principle component analysis (Fig2) showed that the cauliflowers and broccolis formed ‘sub’ genepools which were distinct from the other crop types. This provides evidence supporting the rationale of a foundation set chosen to represent the diversity of the genepool.

Figure 1: Diversity analysis of 94 BoDFFS founder lines

Figure 2: Principal Coordinate Analysis of 100 SSRs on 94 founder lines of B.oleracea DFFS

Originally it was intended to produce ‘fixed’ lines by either microspore culture or by single seed descent. However, because of the need to vernalise many of the founder accessions for 12 weeks to induce flowering, it was decided to concentrate on producing genetically fixed double haploid lines by microspore culture, this was done using a protocol already established at Warwick HRI. A wide range of responsiveness to microspore culture was experienced, reflecting the genetic diversity of the founder lines. In total 79 lines, out of 124 tested, responded to microspore culture to give one or more DH plants and so far seed has been obtained from 50 of these. The yield
of this seed was very variable and during regeneration the seed for 7 lines did not germinate and these lines are now lost. To date we have a total of 43 new fixed lines and are waiting for the remaining microspore-derived plants to flower. In parallel with the microspore DH line production, a programme of single seed descent (SSD) was also being carried out. For the founders with no fixed lines, to date 6 lines have gone through 3 rounds of SSD, 24 lines have been through 2 generations and 49 lines have been through one generation. During the course of the project we have received ‘in kind support’ from a number of breeding companies who are attempting to produce DH lines form the founder accessions of the 3rd and 4th subsets; at the time of writing we have been informed that they have been successful but do not have a definitive list of the lines/seed produced. This resource has been regularly promoted at international meetings and on the internet and the current status of the BolDFFS is made available to stakeholders via the Brassica info website (http://www.brassica.info/resource/plants/diversity_sets.php)

Conclusions and future work: The objective has been partially achieved. The set of founder lines represent the genetic diversity within the *B. oleracea* crop types held in the main gene bank collections and the ‘Genomiphied’ DNA from each parental founder plant is available for future projects and for public distribution. Either founder or fixed lines have been used in a number of projects at WHRI, including water use efficiency, mineral use efficiency and seed oil content.

Producing genetically fixed DH lines from the founder accessions in subsets 1 and 2 has been partially successful and in total 68 genetically fixed lines have been sourced/produced. This work has been supplemented by ‘in kind’ support from breeding companies who are attempting to produce DH lines from the accessions in subset 3 and 4. In the future we do not propose to expend any further effort in fixing additional lines, unless a crop type is significantly under-represented, but will instead construct a ‘pragmatic’ DFFS with the fixed lines available to us and put our efforts in to producing seed to make them available as a public resource and to genotypically characterise them using SNP markers.

2. Regenerate key reference *B. oleracea* DH mapping populations

Introduction: Doubled haploid mapping populations and the associated information (linkage maps and genomic maps associated molecular markers and trait data) are a key resource for the brassica research community and there is a growing demand for them from researchers throughout the UK and the international research community. WHRI curates a number of such populations, but in order for them to be available for use in projects there was an urgent requirement to regenerate the existing seed stocks which were getting old and depleted.

Methods and results: In this project we have regenerated two key reference mapping populations to ensure that they are publicly available; these are the ‘AG’ mapping population consisting of DH lines derived from the cross between the Chinese kale DH line A12 and the broccoli DH line GD33 derived from the F1 hybrid cv Green Duke. Originally it was also intended to regenerate the ‘NB’ mapping population of DH lines derived from a cross between a cauliﬂower DH line derived form the cv Nedcha and an unnamed broccoli inbred line. However, following discussion with the Defra project ofﬁcer (May 2006) it was agreed to replace the regeneration of the NB mapping population with the regeneration of the AG substitution line (AGSL) population which is derived from the same original cross as the AGDH population and has significant value in providing the ability to ﬁne-map QTLs identiﬁed in the AGDH population and are therefore a complementary strategic resource to the AG DH mapping population. For the AGDH population 125 lines were subjected to regeneration attempts, with over 80 lines yielding over 1g seed. Some lines showed signiﬁcant self incompatibility and only small amounts of seed were obtained and 2 lines failed to yield any seed at all. All lines of the AGSL regenerated well with over 5g seed obtained for nearly all the lines. Sufﬁcient seed was produced to make the populations publicly available on a ‘cost recovery basis’

Conclusions and future work: The objective has been fully achieved and these key strategic mapping populations are now publicly available. These 2 populations have been distributed widely and have supported trait-based projects at WHRI in the areas of water use efﬁciency, mineral use efﬁciency, juvenility, seedling vigour, fatty acid and glucosinolate content and virus resistance. Additional genotyping of these populations has also been carried out in BBSRC sponsored research, including the AdVaB project. They have also been distributed internationally to researchers and industry.

We will continue to make them available on a cost recovery basis so that we can ‘finance’ their future regeneration.

3 ‘Capture’ genetic variation present in the secondary genepool of C genome wild species in a form that is utilisable as a strategic resource by breeders and researchers for crop improvement of vegetable brassicas by developing a C genome DFFS.

Introduction: The BolDFFS represents the genetic variation in the major UK brassica vegetables (cabbage, broccoli, cauliﬂower, kale and Brussels sprout) which are the domesticated forms of *Brassica oleracea*. Domestication of *B. oleracea* is unlikely to have captured all of the potentially beneﬁcial alleles in the genepool and in addition to wild *B. oleracea* there are several other wild *Brassica* species which together make up the brassica C genome genepool and which may be potential sources of beneﬁcial alleles. These species are interﬁerte with *B. oleracea* and so any useful genetic variation identiﬁed in them is transferable to vegetable brassicas by conventional breeding techniques. This wider range of genetic variation is not easily accessible for breeders or researchers; there is a high level of within accession variability, seed production is difﬁcult for many accessions and often plants take 3-4 years before they ﬂower and many are self-incompatible and produce few
seeds. The Warwick HRI GRU holds a collection of wild C genome accessions that are not currently available to researchers or breeders due to lack of sufficient seed. In this project we have initiated the production of a DFFS from these ‘wild’ C genome species to complement the BolDFFS described above; this is based on founder lines representing the range of wild C genome species. This DFFS also complements the B. napus DFFS being developed within OREGIN which also encompasses genetic variation in the C genome, as well as the A genome.

**Methods and results:** The strategy used for developing the C-DFFS was different to that adopted for the B. oleracea-DFFS. We have attempted to ‘capture’ genetic variation in a plant form more amenable for genetic studies and utilisation in crop improvement. 99 accessions of ‘wild’ C genome brassicas from the Genetic Resources Unit representing 15 species and were identified as possible members of the C genome Diversity Fixed Foundation Set (DFFS). The initial foundation set was 99 accessions spread as evenly as possible (subject to availability) across 15 wild species (B. alboglabra, B. atlantica, B. balearica, B. bourgaei, B. cretica, B. hilarionis, B. incana, B. insularis, B. montana, B. macrocarpa, B. oleracea, B. rupestris, B. villosa). Seed was sown in April 2004 with the aim of producing 2 plants per line. However, 8 accessions failed to germinate and subsequently the single plant of one accession has died and another was mislabelled. The C genome DFFS therefore consists of 89 lines in total representing 13 species. Up to two plants per accession were grown in a glasshouse. One plant per accession was selected as the ‘founder’ for each fixed line (normally the first plant to flower). This founder plant was crossed to a rapid cycling, self-compatible B. oleracea DH line derived from an AG substitution line which which had few crop characteristics but was known to be responsive to microspore culture and for which good genotype information was available. The founder plant was also selfed as was the ‘second’ plant where available, to produce S1 seed. 15 plants have still not flowered after 5 growing seasons, illustrating the difficulty of exploiting this material. DNA samples were extracted from all plants and secured in the Warwick HRI genomic centre archive, the DNA of the founder plants has been ‘Genomiphied’ for distribution. Microspore culture is being used to generate DH lines from the F1, these will be selected for self compatibility and for ‘rapid flowering’ and also screened for as much of the wild species genotype using SSRs. In this way we will aim to capture as many of the ‘wild species alleles’ as possible in a plant form that is more amenable for genetic and phenotype analysis. Unlike the B. oleracea-DFFS where only one fixed line will be produced per foundation accession, it is necessary to select a small number of DH lines derived from each C genome accession in order to capture as much genetic variability as possible. Leaf samples of all F1 plants used for microspore culture have been secured at -80°C. To date F1s from 74 accessions (from 12 species) have responded to microspore culture, however, the number of spontaneous diploids has been relatively low for many accessions and seed production has been difficult. To date seed has been produced from 46 DH plants derived from accessions representing 10 species. The current status of the CDFFS is made available to stakeholders via the Brassica info website (http://www.brassica.info/resource/plants/diversity_sets.php).

The diversity within the C DFFS founder lines was originally planned to be assessed by screening with 3 mapped SSRs per linkage group, however, because of the diversity within the founder lines some SSRs did not produce useful data and the number of SSRs finally used was expanded to 48.

**Table 1:** Number of alleles identified in 79 C genome DFFS founder lines using 3 SSRs mapped to LG4

<table>
<thead>
<tr>
<th>No. accessions</th>
<th>No. alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brassica oleracea</td>
<td>21</td>
</tr>
<tr>
<td>Brassica macrocarpa</td>
<td>16</td>
</tr>
<tr>
<td>Brassica cretica</td>
<td>8</td>
</tr>
<tr>
<td>Brassica villosa</td>
<td>9</td>
</tr>
<tr>
<td>Brassica rupestris</td>
<td>4</td>
</tr>
<tr>
<td>Brassica montana</td>
<td>3</td>
</tr>
<tr>
<td>Brassica incana</td>
<td>10</td>
</tr>
<tr>
<td>Brassica alboglabra</td>
<td>1</td>
</tr>
<tr>
<td>Brassica insularis</td>
<td>2</td>
</tr>
<tr>
<td>Brassica bourgaei</td>
<td>1</td>
</tr>
<tr>
<td>Brassica hilarionis</td>
<td>2</td>
</tr>
<tr>
<td>Brassica atlantica</td>
<td>2</td>
</tr>
</tbody>
</table>
Figure 3: Principal Coordinate Analysis of 48 SSRs on 79 founder lines of C genome DFFS

The diversity found in the CDFFS founder lines is significantly greater than the diversity found in the BolDFFS founder lines. Screening with 3 SSR markers across a single linkage group identified a large number of alleles. (Table 1) and screening with the 48 SSR markers across all LGs identified a total of 4357 alleles. Diversity analysis of this data for the 79 accessions that have flowered and been crossed to B oleracea, showed the accessions largely grouping together by species. Principal component analysis (Fig 3) showed the species forming sub genepools, particularly B. macrocarpa.

Conclusion and further work: The objective has only been partially achieved; the lengthy time taken for many of the plants has been greater than anticipated delaying completion of this objective. We have evidence from diversity screening in this project and also allelic surveys for specific genes in other projects that the wild C genome species represent a significant resource of genetic diversity greater than that found within the domesticated genepool. We therefore, propose to continue with efforts to capture the alleles present in the wild C genome species as these represent a potential source of novel alleles for use in a 'targeted domestication' pre-breeding programme for both horticultural brassicas and the oil seed rape crop. We will therefore continue with efforts to produce and seed DH lines from the F1s between the various wild species and the common oleracea parent. However, because we anticipate difficulties in producing seed from some of the resultant DH lines we will also produce F2 seed lots from each cross. Although these will be segregating and will be of limited value for phenotypic assessment they will 'conserve' beneficial alleles which may be identified through targeted molecular screens for allelic diversity of the founder DNA samples. The F2s will have the added advantage over the parental accessions of segregating for flowering time, i.e. some plants with beneficial alleles will flower earlier than the wild parent accession (and similarly will segregate for self compatibility so that seed production is less problematic. Both of these traits will make for easier access to the beneficial alleles for breeding.

4. Transfer resistance to Xanthomonas campestris pv campestris from B. rapa to B. oleracea
Introduction: Work under this objective was to complete research initiated in HH0910SFV to introgress resistance to Xanthomonas campestris pv campestris (X.c.c.) from B. rapa to B. oleracea. X.c.c. is considered to be the most important disease of horticultural brassicas world-wide and is becoming increasingly important in the UK. However, there appears to be little useful resistance for X.c.c within the B. oleracea. We have previously identified a B. rapa source of resistance to the 2 most important races of X.c.c.races 1 & 4 and synthetic amphidiploids (AACC= B.napus) resistant to races 1 and 4 were made using embryo rescue techniques. The resistance to both races was shown to be quantitative and prior to the start of this project we had identified 4 putative QTLs for resistance.

Methods and results: The Brassica rapa genetic map based on the F2 between the susceptible rapid cycling inbred line Ro18 and the source of resistance B162 was consolidated into 10 linkage groups covering 722 cM and linked to the B. rapa reference genetic map (8). QTL analysis of this map confirmed 3 QTL that accounted for most of the variance for resistance: a large QTL for resistance to both XCC race 1 & race 4 on linkage group 8, an additional QTL on group 8 for race 4 resistance and a QTL for race 1 resistance on LG 10. These results were
published in Soengas et al, 2007 (4). The QTL analysis identified AFLP markers suitable for marker assisted selection for *X.c.c.* resistance during a backcross programme aimed at introgressing the resistance from the A genome to the C.

Residual seed from the amphidiploids were tested for resistance to races 1 and 4 using a standard pathology assay (4) and resistant plants were kept for selfing and backcrossing to the *B oleracea* DH line AG 1011 as male parent (no embryos were recovered using AG1011 as the female.) Embryo rescue was used to obtain the BC1 plants, which were confirmed by flow cytometry, attempts to self BC1 plants failed because of male sterility and so they were backcrossed again. The resistance of BC1 plants was tested under glasshouse conditions. Tests were carried out on cuttings taken from selected plants of BC1 because the BC1 plants were male sterile and were required for further backcrossing; three cuttings were taken from 5 plants of the BC1 generation. Plants were tested with races 1 and 4 of *Xcc*. The results between plants and cuttings were variable and the plants were susceptible or only partially resistant. Two BC1 plants had a partial level of resistance to race 1. Marker assisted selection was used to identify 8 putative resistant BC2 lines; three cuttings were taken from each plant for testing for resistance. Three plants showed a partial level of resistance and these were backcrossed again. BC3 plants were produced mainly by embryo rescue although two plants were produced without intervention. BC3 plants were confirmed by flow cytometry as possessing an amount of DNA similar to *B oleracea* controls. Molecular analysis confirmed that QTL for resistance were present in one BC3 plant but this was male sterile and it was not possible to produce any self pollinated seed form it. At this stage (yr 4 of the project) it was agreed to stop this work as resources were needed for developing the C DFFS.

**Conclusion and further work:** The objective was not achieved in that the QTL for resistance to *X.c.c.* were not introgressed from *B rapa* to *B oleracea*. However, the work laid the foundations for a follow up 4 year BBSC/DfID SARID (Sustainable Agriculture for International Development) project - Accelerated breeding of black rot resistant brassicas for the benefit of East African smallholders. This has the objective of identifying the genes underlying the QTL for resistance for a dual approach of using introducing resistance to *B oleracea* through transgenics and searching for similar genes in the C genome DFFS through molecular screening.

5. Release breeding lines with resistance to *Brevicoryne brassicae* to breeding companies:

**Introduction:** Work under this objective was to complete research initiated in HH0910SFV to produce breeding material with resistance to the cabbage aphid (*Brevicoryne brassicae*) derived from accessions of the C genome wild species *B. cretica* and *B. incana* and to release this material to commercial brassica breeding companies.

**Methods and results:** Microspore culture and regeneration of the *B. cretica* x *B. oleracea* line Senna (CxS) and *B. incana* x Senna (IxS) F1s continued until year 2 of this project. Culturing of IxS F1s produced 62% spontaneous DH lines whereas the CxS F1s produced 48% DH lines. However, regeneration/germination of the IxS embryos was far more difficult than the CxS embryos and in total 423 CxS DH lines and 111 IxS DH plantlets were produced. Losses occurred at the weaning stage and seed production was difficult particularly for the IxS DH lines where only 10 out of 59 DH plants produced any seed. Seed production from the CxS plants was more successful with 68 out of 187 DH plants producing seed. The 78 DH lines were tested for resistance to cabbage aphid in the insect rearing unit at Warwick HRI.

**Table 2:** Summary of analysis of variance of DH lines derived from *Brassica cretica* and *B. incana* for numbers of apterae, nymphs and total number of cabbage aphid (*Brevicoryne brassicae*)

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Nymphs</th>
<th>Apterae</th>
<th>Total aphids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line MS</td>
<td>77</td>
<td>2.112</td>
<td>1.5981</td>
<td>1.988</td>
</tr>
<tr>
<td>Residual MS</td>
<td>276 (32 MV)</td>
<td>1.228</td>
<td>0.9582</td>
<td>1.135</td>
</tr>
<tr>
<td>VR</td>
<td>1.72 p&lt;.001</td>
<td>1.67 p =0.002</td>
<td>1.75 p &lt;.001</td>
<td></td>
</tr>
</tbody>
</table>

Analysis of variance showed that there were significant differences between the lines for numbers of nymphs, *apterae* and total number of aphids (Table 2) and several lines performed significantly better than the Brussels Sprout control. The best five lines were released to a consortium of breeding companies and included in further tests carried in a confidential project funded by the three breeding companies to identify sources of resistance to brassica pests.

**Conclusion and further work:** The objective was achieved. Three of the 5 lines released to the breeding companies continue to show a significant level of resistance in replicated experiments demonstrating the effectiveness of producing DH lines from wild C genome species crossed with *B. oleracea* when attempting to identify sources of beneficial alleles determining complex quantitative traits. This has significance for the production of the C DFFS.

6. Determine whether monogenic resistance to downy mildew derived from different sources are allelic or distinct from one another

**Introduction:** Sources of downy mildew (*Hyaloperonospora parasitica* subsp. *brassica*; *Hpb*) resistance were identified as part of a previous EU project (‘The location and exploitation of genes for pest and disease resistance in European gene bank collections of horticultural brassicas’ from 1993 to 1997). In a subsequent project funded...
by Defra on ‘Variability of fungal pathogens’ (2002/03), resistant plants of the selected accessions were crossed
with rapid cycling plants and self compatible, double haploid (DH) lines were obtained from the F₁s via microspore
culture. Three DH lines were broadly resistant to a collection of 43 UK Hpb isolates. Further genetics and
breeding studies were carried out in the current project to determine whether the resistances were allelic or
distinct.

Methods and results: The three different sources of broad spectrum resistance to Hpb studied in this project
were:

- DH525 (OL2035) – derived from a Borecole
- DH544 (OL2036) – derived from a Summer cabbage
- DH550 (OL2060) – derived from a Borecole

Six crosses were made between the sources of resistance.

- DH525 x DH544 (and reciprocal)
- DH525 x DH550 (and reciprocal)
- DH544 x DH550 (and reciprocal)

The resultant F₂s were then self-pollinated to produce F₃s.

In addition, plants of the three resistant lines and of differential lines identified in the previous project have been
self-pollinated to generate seed to be used in future experiments.

A core collection of 25 single spore H. parasitica isolates has been established. The isolates were
selected from isolates obtained in the previous Defra funded project. All 25 isolates have been multiplied on kale
cv Maris Kestrel and infected cotyledons have been frozen at -80°C for long term maintenance. A duplicate
collection has been placed in the Warwick HRI freezer archive. The 25 single spore isolates together with the two
isolates originally used to identify the sources of resistance (Hp005 and 006) were tested on 20 accessions
including resistant, susceptible and differential lines (Figure 4). The results confirmed that the three DH lines
(DH525, 544, 550) were broadly resistant to the Hpb isolates tested; three DH lines (DH502, 527, 508) displayed
a differential responses to the Hpb isolates tested.

![Figure 4. Results of pathogenicity tests of 27 Hpb isolates in 17 Brassica oleracea accessions and three
B. napus accessions (N-o-1, N-o-9, Cob76).](image)

A series of tests for allelism were performed on the F₂ lines. Over 3200 F₂ plants were tested in total (at least 500
F₂ plants per cross) with one Hyaloperonospora parasitica isolate (Hp717) together with control plants of the F₁,
parental lines and a susceptible control (Senna). There was no evidence of segregation in the F₂s derived from
crosses between the three sources of resistance (Table 3) and therefore we conclude that the potential broad
spectrum resistance to Hpb derived from three different plant genetic resource accessions is controlled by the
same gene/locus or closely linked genes/loci.

<table>
<thead>
<tr>
<th>Line</th>
<th>Derived from cross</th>
<th>Total tested</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₂ - JV06001</td>
<td>DH525 x DH544</td>
<td>523</td>
<td>Resistant</td>
</tr>
<tr>
<td>F₂ - JV06006</td>
<td>DH544 x DH525</td>
<td>592</td>
<td>Resistant</td>
</tr>
<tr>
<td>F₂ - JV06025</td>
<td>DH550 x DH544</td>
<td>502</td>
<td>Resistant</td>
</tr>
<tr>
<td>F₂ - JV06020</td>
<td>DH544 x DH550</td>
<td>520</td>
<td>Resistant</td>
</tr>
<tr>
<td>F₂ - JV06011</td>
<td>DH525 x DH550</td>
<td>531</td>
<td>Resistant</td>
</tr>
<tr>
<td>F₂ - JV06016</td>
<td>DH550 x DH525</td>
<td>540</td>
<td>Resistant</td>
</tr>
</tbody>
</table>
In order to further assess the value of the resistance in controlling downy mildew it was necessary to challenge the resistance with a broader range of isolates representing the current UK population of H. parasitica. Samples of leaves or cotyledons of B. oleracea plants were collected from plant raising nurseries in major growing areas of the UK (Table 4). Samples of downy mildew infected leaf tissue were washed with 2-3 ml of distilled water and the resulting suspension was drop inoculated onto 9-day-old kale Maris Kestrel seedlings. The inoculated seedlings were then incubated for 7 to 14 days at 15°C with a day length of 12 hours. Cotyledons with sporulation were harvested and washed and the spore suspension was inoculated again into kale seedlings. After 7 days incubation, some cotyledons were used to make a suspension for a pathogenicity/spectrum of resistance test and the remainder cotyledons stored at –80°C.

Table 4. Origin of new Hyaloperonospora parasitica (HpB) isolates

<table>
<thead>
<tr>
<th>Number</th>
<th>County</th>
<th>Plant nursery</th>
<th>Cultivar</th>
<th>Date of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>801</td>
<td>Somerset</td>
<td>Wessex</td>
<td>Pavilion</td>
<td>19/02/2007</td>
</tr>
<tr>
<td>802</td>
<td>Lincolnshire</td>
<td>Westhorpe</td>
<td>Duchy</td>
<td>03/12/2007</td>
</tr>
<tr>
<td>803</td>
<td>Lincolnshire</td>
<td>Westhorpe</td>
<td>Tinman</td>
<td>03/12/2007</td>
</tr>
<tr>
<td>804</td>
<td>Lincolnshire</td>
<td>Westhorpe</td>
<td>Marathon</td>
<td>03/12/2007</td>
</tr>
<tr>
<td>805</td>
<td>Lancashire</td>
<td>Farringtons</td>
<td>Boris</td>
<td>30/01/2008</td>
</tr>
<tr>
<td>806</td>
<td>Lancashire</td>
<td>Farringtons</td>
<td>Helsinki</td>
<td>30/01/2008</td>
</tr>
<tr>
<td>807</td>
<td>Lancashire</td>
<td>Farringtons</td>
<td>Trent</td>
<td>30/01/2008</td>
</tr>
<tr>
<td>808</td>
<td>Lincolnshire</td>
<td>Sheepgate</td>
<td>Pavilion</td>
<td>08/04/2008</td>
</tr>
<tr>
<td>809</td>
<td>Lincolnshire</td>
<td>Sheepgate</td>
<td>Concept</td>
<td>08/04/2008</td>
</tr>
<tr>
<td>810</td>
<td>Lincolnshire</td>
<td>Sheepgate</td>
<td>Valtos</td>
<td>08/04/2008</td>
</tr>
<tr>
<td>811</td>
<td>Lincolnshire</td>
<td>Sheepgate</td>
<td>Kronas</td>
<td>08/04/2008</td>
</tr>
<tr>
<td>812</td>
<td>Lincolnshire</td>
<td>Sheepgate</td>
<td>Valtos</td>
<td>08/04/2008</td>
</tr>
<tr>
<td>813</td>
<td>Lincolnshire</td>
<td>Sheepgate</td>
<td>Concept</td>
<td>08/04/2008</td>
</tr>
<tr>
<td>814</td>
<td>Lancashire</td>
<td>Farringtons</td>
<td>Calabrese cv.?</td>
<td>02/04/2008</td>
</tr>
<tr>
<td>815</td>
<td>Lancashire</td>
<td>Farringtons</td>
<td>Calabrese cv.?</td>
<td>02/04/2008</td>
</tr>
</tbody>
</table>

Nine day-old seedlings of accessions derived from the three sources of resistance together with controls were inoculated with fifteen isolates HpB 801 to 815. (Fig 5.) The DH525, 544 and 550 lines were susceptible to two Lancashire isolates. The DH502 line was partially resistant to the isolates from Lancashire. In addition, the DH lines have been tested with two Portuguese isolates by Dr Paula Coelho and Luísa Valério in Professor António Monteiro’s laboratory at the Instituto Superior de Agronomia, Lisbon, Portugal. The lines DH525, 544 and 550 were susceptible to two of the Portuguese isolates. The DH527 line was resistant to these isolates.

Conclusions and future work: The objective was achieved; the three sources were shown to be allelic. However, although the initial testing against 25 single spore isolates indicated the resistance might be broad spectrum subsequent testing against the collection of UK isolates showed that the resistance could be overcome. We are continuing the collaboration with Professor Monteiro's team and are testing selected lines from Portugal with UK isolates and selected lines bred in the UK are being tested with Portuguese isolates in Lisbon.

Figure 5. Results of pathogenicity tests of 15 HpB isolates in 15 Brassica oleracea accessions and three B. napus accessions (N-o-1, N-o-9, Cob76).
7. Introgress at least 2 sources of downy mildew resistance into a crop phenotype

Introduction: The resistant DH lines described in 6 above have no recognisable crop morphology. The objective was to introgress resistance into a recognisable crop phenotype such as broccoli to provide experimental lines for future experiments aimed at integrating host resistance with other control measure to provide sustainable disease control at the crop level.

Methods and results: Crosses were made between two sources of resistance (DH525 and DH550) and a DH calabrese line (CAL18b) and an open pollinated mini-veg cauliflower (cv. Lateman). The F1, F2, BC1 generations

Table 5: Segregation of resistance to *Hyaloperonospora parasitica* in the F2 and BC1 generations derived from crosses between the resistant accession DH525 and a broccoli (CAL18b) or cauliflower line (Lateman)

<table>
<thead>
<tr>
<th>Line</th>
<th>Derived from cross</th>
<th>Result</th>
<th>Proposed segregation</th>
<th>X²</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH525</td>
<td>Resistant parent</td>
<td>9 res</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal 18b</td>
<td>Susceptible parent</td>
<td>8 sus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Senna</td>
<td>Susceptible control</td>
<td>8 sus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>DJ9011 x 525</td>
<td>22 res</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BC1</td>
<td>DJ9011 x F1</td>
<td>50 res : 37 sus</td>
<td>1 : 1</td>
<td>0.16</td>
</tr>
<tr>
<td>F2</td>
<td>Self F1</td>
<td>133 res : 45 sus</td>
<td>3 : 1</td>
<td>0.93</td>
</tr>
<tr>
<td>DH525</td>
<td>Resistant parent</td>
<td>7 res</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateman</td>
<td>Susceptible parent</td>
<td>10 sus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Senna</td>
<td>Susceptible control</td>
<td>9 sus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>525 x Lateman</td>
<td>11 res</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BC1</td>
<td>F1 x Lateman</td>
<td>45 res : 38 sus</td>
<td>1 : 1</td>
<td>0.44</td>
</tr>
<tr>
<td>F2</td>
<td>Self of F1</td>
<td>136 res : 43 sus</td>
<td>3 : 1</td>
<td>0.76</td>
</tr>
</tbody>
</table>

from these crosses were tested with one isolate of *Hyloperonospora parasitica* (HpB 717). As expected the BC1 generations were segregating approx. 1:1 and the F2 generations approx. 3:1 (Tables 5 and 6) which confirmed that the resistance of both DH525 and DH550 are controlled by one gene/locus. Some resistant plants of the BC1 generation were selected on their morphology to be further back-crossed.

However, in a second approach to produce genetically fixed lines, F1 plants derived from crosses of the two resistant lines with a the DH calabrese line CAL18b were microspore cultured and 45 DH plants were produced. A leaf strip assay to test the plants with one isolate of *H. parasitica* gave unreliable results, so all the plants were self pollinated and the self-progeny seedlings were tested.

Table 6. Segregation of resistance to *Hyaloperonospora parasitica* in the F2 and BC1 generations derived from crosses between the resistant accession DH550 and a broccoli (Cal 18b) or cauliflower line (cv Lateman)

<table>
<thead>
<tr>
<th>Line</th>
<th>Derived from cross</th>
<th>Number tested</th>
<th>Proposed segregation</th>
<th>X²</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH550</td>
<td>– Resistant parent</td>
<td>10 res</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal 18b</td>
<td>Susceptible parent</td>
<td>9 sus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Senna</td>
<td>Susceptible control</td>
<td>9 sus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>Cal 18b x 550</td>
<td>10 res</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BC1</td>
<td>Cal 18b x F1</td>
<td>43 res : 35 sus</td>
<td>1 : 1</td>
<td>0.36</td>
</tr>
<tr>
<td>F2</td>
<td>Self F1</td>
<td>119 res : 50 sus</td>
<td>3 : 1</td>
<td>0.17</td>
</tr>
<tr>
<td>DH550</td>
<td>Resistant parent</td>
<td>11 res</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateman</td>
<td>Susceptible parent</td>
<td>9 sus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Senna</td>
<td>Susceptible control</td>
<td>10 sus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>544 x Lateman</td>
<td>24 res</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BC1</td>
<td>F1 x Lateman</td>
<td>47 res : 42 sus</td>
<td>1 : 1</td>
<td>0.60</td>
</tr>
<tr>
<td>F2</td>
<td>Self of F1</td>
<td>127 res : 52 sus</td>
<td>3 : 1</td>
<td>0.21</td>
</tr>
</tbody>
</table>

36 plants produced sufficient seed for testing and half were resistant. Five DH lines showed an intermediate phenotype or partial susceptibility, with confined sporulation and some necrotic flecking (Table 7). Four resistant DH lines were selected for morphology and self compatibility to be multiplied.
Table 7: Result of progeny test of DH lines derived from crosses between Cal 18b and DH525 or DH550

<table>
<thead>
<tr>
<th>F₁ used for microspore culture</th>
<th>Diploid plants that survived</th>
<th>Progeny tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>JV05026 (Cal 18b x DH525)</td>
<td>37</td>
<td>17 res: 5 partially susc : 11 susc</td>
</tr>
<tr>
<td>JV05022 (Cal 18b x DH550)</td>
<td>8</td>
<td>1 res: 2 susc</td>
</tr>
<tr>
<td>Totals</td>
<td>45</td>
<td>18 res: 5 part susc: 13 susc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18 res: 18 susc (1:1)</td>
</tr>
</tbody>
</table>

Conclusions and future work: The objective has been achieved and the two sources of resistance have been introgressed into a crop type phenotype. This material is now available for experiments aimed at integrating host resistance with other forms of disease control such as disease forecasting being developed at Warwick HRI. Plants of the back cross generation (BC₁) derived from crosses between the Cal 18b and the three resistance sources have been supplied for microspore culture.

8: Identify new sources of downy mildew resistance in a foundation set of wild C-genome species (S₁ generation) and capture the resistance by transferring it into a rapid cycling background

Introduction: Strategies for more sustainable deployment of host resistance have been successfully implemented in cereals, such as the use of cultivar mixtures or multi-lines (constituent lines differing only in resistance). A strategy for deploying resistance to *Hpb* to provide sustainable downy mildew control in vegetable brassica requires additional sources of resistance. Previous EU and DEFRA funded projects found that genetic variation for downy mildew resistance in *B. oleracea* is limited and results from this project have shown that the 3 sources of resistance that were identified are allelic (see section 6 above). This lack of variation may be due to a narrowing of the genetic base during domestication. The C genome diversity set assembled in this project is a resource which can be screened for additional sources of resistance.

Methods and results: A total of 74 S1 lines derived by self pollination of the CDFFS founder plants were tested for resistance to a single isolate of *Hyaloperonospora parasitica* (HpB 717). Most lines were very susceptible and no lines contained plants that were totally resistant but some lines had plants which displayed partial resistance (Table 8).

Table 8. Screening of C genome accessions with one *Hyaloperonospora parasitica* isolate (HpB 717)

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of lines tested</th>
<th>Number of lines with</th>
<th>Number of lines with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>All plants susceptible</td>
<td>Some plants with partial resistance</td>
</tr>
<tr>
<td>Brassica alboglabra</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Brassica atlantica</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Brassica bourgaei</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Brassica cretica</td>
<td>7</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Brassica hilarionis</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Brassica incana</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Brassica insularis</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Brassica macrocarpa</td>
<td>16</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Brassica oleracea</td>
<td>21</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>Brassica montana</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Brassica rupestris</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Brassica villosa</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Totals</td>
<td>74</td>
<td>63</td>
<td>11</td>
</tr>
</tbody>
</table>

Conclusions and future work: The objective has been partially achieved. Although no sources of complete resistance were identified, putative sources of partial resistance were found. Given the apparent breakdown of the ‘dominant major gene resistance’ reported in 6 above, partial resistance may be more durable, however, this will have to be tested and a sub-set of the accessions will be tested with at least three more isolates. Given the length of time needed to produce the S1 seed from the C DFFS founder plants (see 3 above) it has not been possible to ‘capture the resistance by transferring it into a rapid cycling background’, however, plants that were partially resistant to HpB 717 have been selected for selfing and further crosses.
9. Identify if pathogenicity factors are common between *Brassica* and *Arabidopsis* isolates of *Peronospora parasitica* (downy mildew) and assess if these can be used to screen for resistance in the C genome foundation set

Work to deliver this objective was carried out as a PhD studentship within the project. Space constraints prevent a detailed description of the work carried out which has been published as a PhD thesis (5). The summary of the thesis is reproduced below.

*Hyaloperonospora parasitica*, phylum Oomycota, is a biotrophic plant pathogen that causes downy mildew on its cruciferous host plants. Naturally-infected plants show disease resistance visible as a hypersensitive response (HR). This response results from the interaction of pathogen effectors with receptors and signalling molecules in the plant cell, causing localised necrosis killing the infected host cell. *H. parasitica* isolates that are host-specific on the model plant *Arabidopsis* have been used to identify such pathogen effector genes. In this work I attempted to use this information to identify similar genes in isolates host specific to the crop plant *Brassica oleracea*.

Potential effector molecules, encoding amino-terminal signal peptides, produced by *Arabidopsis* isolates of *H. parasitica* were sequenced in up to 16 isolates and analysed for selection pressure, using population genetic methods. Highly variable genes were categorised as candidate virulence factors that might be involved in molecular interactions with the host detection machinery. Since they exhibit high amino acid variability, they are likely to be under positive selection pressure for change. The most diverse gene (*ATR13*) was cloned and expressed in planta to test for HR. This group of genes were then used to determine if they were under similar levels of selection in *B. oleracea* isolates of *H. parasitica*. To search for the homologues in this related *H. parasitica* subspecies a genomic library and an expressed sequence tag (EST) library were produced, as direct methods of gene isolation and were generally unsuccessful. The two main strategies employed were the use of microsyntenies to localise effector regions in the genome and a random sequencing approach to search for genes that encode signal peptides and effector motifs, such as the predicted targeting motifs RXLR and DEER, which are important to the delivery of oomycete effectors into host cells. While it was possible to find the regions of *ATR13* and *ATR1* in the genome, it was not possible to find the orthologues of these avirulence factors. Using the EST approach many new candidate effectors were identified. It is clear from the data produced in this project that although the *H. parasitica* isolates from *B. oleracea* and *Arabidopsis* both infect *Brassicaceae*, they are very distantly related. So far no highly variable genes were found in the crop pathogen, although many interesting new candidates for secreted virulence factors were identified and work on a potential locus of avirulence factors has been initiated.

10. Identify loci for pathotype-specific and broad-spectrum resistance to TuMV in the *Brassica* A genome to determine whether any are amenable to transfer to the C genome

**Introduction:** *Turnip Mosaic Virus* (TuMV) is an important pathogen of brassica vegetables throughout the world (6). It causes far greater crop losses in the UK than in many other parts of northern Europe. Currently there is a very restricted range of approved insecticides for use on horticultural crops for controlling the aphid vectors of TuMV. None of those insecticides that are available will reduce primary infection of crops by TuMV, since it is transmitted in a non-persistent manner. Host resistance is therefore the most viable route for controlling this virus.

**Methods and results:** The extreme resistance of the *Brassica napus* line 165 to *Turnip mosaic virus* (TuMV) pathotypes 1 and 3 was established. Following challenge of line 165 plants with TuMV isolates belonging to these two pathotypes, no visible symptoms were seen and no virus replication was detectable. Line 165 was crossed with a TuMV susceptible *B. napus* line to produce F1 plants that were resistant to TuMV. An F1 individual was backcrossed to the susceptible *B. napus* line to produce a BC1 mapping population. A genetic map based on 286 marker loci segregating in 46 BC1 individuals was produced and aligned with existing *B. napus* maps. The 46 BC1 plants were self-pollinated to produce BC2S1 families that were tested for resistance to different TuMV isolates.

Analysis of the data identified resistance was controlled by a single locus named *Turnip mosaic virus Resistance in Brassica 04* (*TuRB04*), which mapped to the lower portion of chromosome N8. This region of the brassica A genome does not appear to be have a high degree of recombination with the C genome. A range of *B. rapa* lines were tested for resistance to TuMV in order to identify other genes for resistance which might be more amenable to transfer to the C genome.

Plants of the *B. rapa* cv K1 previously found to possess novel TuMV resistance were selfed and crossed to a rapid cycling TuMV-susceptible *B. rapa* line R-o-18. Selfed progeny of K1 exhibited broad-spectrum resistance to TuMV. None of the isolates (representing pathotypes 1, 3 and 4) induced any infection. Five F1 lines were tested for resistance to the sum of TuMV isolates. Dominant resistance to UK 1 (pathotype 1) and CZE 1 (pathotype 3) was found in one F1 line and dominant resistance to GBR 6 (pathotype 4) was found in three of the F1 lines. A BC2 population for mapping the dominant resistance was produced, phenotyped and a linkage map for the population is being constructed.

As a sub-set of the *B. rapa* core collection from the GRU at Warwick HRI was also selected for testing for resistance to TuMV. Twenty one lines were tested separately against TuMV isolates UK 1 (pathotype 1) and GBR 6 (pathotype 4; no dominant sources of resistance to GBR 6 had been identified at that time). Resistance was seen in 5 lines and plants were kept and grown on for selfing and crossing to a rapid cycling TuMV-susceptible *B. rapa* line. Where resistance to UK 1 was seen, some plants were re-inoculated with an infectious clone of TuMV.
(vVIR24) in which a single nucleotide had been changed such that the isolate overcame the mapped resistance gene TuRB01 (to determine whether the resistance was due to the previously mapped resistance gene TuRB01). Where resistance to GBR 6 was seen, some plants were re-inoculated with TuMV isolate UK 4 (to determine whether the resistance was also effective against pathotype 12; no such dominant resistance had been found at that time). One line showed resistance to both vVIR24 and UK 4, one showed resistance to UK 4, one gave necrosis with vVIR24 and one showed resistance to vVIR24, but not GBR 6. For two of the accessions in which resistance was identified, there was only one resistant plant. The plant from one of these accessions died before producing seed and the plant from the other accession only produced a small amount of seed which had poor germination. For another accession there were two resistant plants one of which was selfed and the other crossed to R-o-18. Very little seed was produced from selfing or crossing. Resistant plants from the other two accessions were successfully selfed and crossed to R-o-18. Two F1 lines, one from a B. rapa campestris parent and the other from a B. rapa rapa parent, were tested for their resistance to a range of TuMV isolates.

The Brassica rapa rapa line displayed resistance to 7 isolates (including UK 1, CHN 5, CZE 1, CDN 1, GBR 6, POL 1 and UK 4) representing the major pathotypes of TuMV. Resistant plants showed no symptoms following mechanical inoculation with TuMV and no virus was detected in the plants by ELISA. A cross was made between R-o-18 and a plant from the resistant B. rapa rapa line. The small amount of the F1 generation seed available from this cross was inoculated with the seven TuMV isolates. F1 plants were uniformly resistant to the UK 1 isolate of TuMV, uniformly susceptible to the CHN 5 isolate (only 2 plants inoculated) and segregated for resistance and susceptibility to the other five TuMV isolates. This suggested that the parent B. rapa rapa plant used in the cross was probably homozygous for one, or more dominant resistance genes to the UK 1 isolate of TuMV and heterozygous for one, or more dominant resistance genes to the other TuMV isolates. When self seed (S1) from the parent plant from the resistant line was inoculated with the TuMV isolates GBR 6 and UK 4, the segregation for the former isolate was not significantly different from 3 resistant to 1 susceptible, whereas for the latter isolate, the segregation was 4 resistant to 9 susceptible, suggesting resistance to GBR 6 is controlled by a single dominant gene, whereas resistance to UK 4 is controlled by two or more dominant resistance genes. The putative resistance genes appear to confer hitherto unknown dominant TuMV resistance specificities. The genetics of the resistance has been written up and published (7).

The F1 plants derived from the cross between B. rapa campestris and R-o-18 segregated for resistance to TuMV isolates (representing pathotypes 1, 3, 4 and 12) and were uniformly susceptible to other TuMV isolates, suggesting the presence of both dominant and recessive resistance genes in the resistant parent (a proportion of F1 plants were resistant to some TuMV isolates, whereas some isolates infected all F1 plants tested). Some F1 plants were back-crossed to plants from the B. rapa campestris parental line and also selfed to produce B1 and F2 seed. Other F1 plants were back-crossed to R-o-18 and also selfed to produce B1 and F2 seed for further evaluation. Phenotyping of S1 plants derived from the resistant parent plant showed that they were uniformly resistant to pathotype 1 (UK 1) and pathotype 4 (GBR 6) isolates of TuMV, suggesting the resistant parent plant was homozygous for both dominant and recessive resistance genes.

Further BC1S1 families derived from a cross between the recessive broad-spectrum resistance source, B. rapa line RLR22 and the susceptible line R-o-18 were characterised. A genetic map based on 213 marker loci segregating in 120 first back-cross (B1) individuals was established and the existence and position of two loci controlling resistance (based on the segregation for resistance and susceptibility in the BC1S1 families) was confirmed (Fig. 6). The first gene, retr01, had a recessive allele for resistance and was located on the upper portion of chromosome R4 (Fig. 6). The second gene, ConTR01, had a dominant allele for resistance and was located on the upper portion of chromosome R8 (Fig. 6). The work has been written up and published (8).

Seed of wild Brassica rapa from Abingdon in Oxfordshire was obtained from CEH Oxford, grown on and selfed. Progeny were obtained from 4 plants, these were grown on and tested for susceptibility to the UK 1 isolate Of the 4 lines, 3 were completely susceptible and one segregated for immunity (no symptoms seen and no virus detected) and susceptibility (1:1). Four immune plants were crossed to R-o-18 and attempts were made to produce self seed also. F1 plants produced from this cross segregated for resistance to UK 1 suggesting that one or more dominant genes were involved in the resistance. F1 plants were also inoculated with vVIR24 (to determine whether the resistance was due to TuRB01) and vVIR151 (to determine whether the resistance was due to TuRB06). Results suggested that neither TuRB01 nor TuRB06 were present in the wild B. rapa and consequently the resistance genes present may be new and worthy of mapping. Three F1 plants from the cross were selfed and back-crossed to R-o-18 to produce F2 and BC1 seed. Very little BC1 seed was produced, consequently, five BC1 plants from each of the three crosses were selfed to determine which produced the most B1S1 seed and hence would be suitable for mapping of the TuMV resistance gene(s).

Conclusion and Future work: The objective has been achieved. Significant progress has been made in mapping the novel dominant broad-spectrum resistance to TuMV in the Brassica rapa line K. Further work is needed to map the genes to particular chromosomes and hence develop molecular markers and determine their potential for transfer to the B. oleracea genome by non-transgenic means. Other sources of resistance to TuMV have been identified in B. rapa that would warrant further characterisation and mapping.
11. Identify new sources of TuMV resistance derived from wild C-genome species

Thirty five S₁ lines of wild *Brassica* spp derived from the founder lines of the Warwick HRI C DFFS were tested for resistance to three TuMV isolates (UK 1, CZE 1 and CDN 1), representing pathotypes 1, 3 and 4. All lines were susceptible to the UK 1 and CDN 1 isolates and three of the lines appeared to be uniformly resistant to CZE 1. Pathotype 1 isolates are the most common occurring in the UK.

12. Complete a QTL analysis of shelf life in broccoli

**Introduction**: We have previously shown that shelf life in broccoli is influenced by genotype (HH0910). Thus opens up the possibility of producing varieties that are less sensitive to environmental influences and therefore provide a more consistent product for the consumer. Work in this project was aimed at providing commercial broccoli breeders with the tools and information needed to breed cultivars with extended shelf life. A mapping population of DH lines derived from a cross between two broccoli DH lines (Mar34 and GD33) which have previously been shown to differ significantly for their shelf life was developed during a PhD project at Warwick HRI. This was used to carry out an initial QTL analysis which identified 3 QTL affecting post harvest discolouration in broccoli. However, the analysis was limited because the population was relatively small (60 DH lines) and the map used for the analysis had significant gaps.

**Methods and results**: In order to improve the genetic analysis of shelf life in broccoli we needed to increase the number of DH lines in the Mar34 x GD33 broccoli mapping population and to improve the coverage of the linkage map.

More DH mapping lines were produced by microspore culture of the F₁ Mar34 x GD33 during yrs 1 and 2 of the project. The F₁ was quite responsive (giving between 300-1000 embryos per 60 buds on most occasions) but it was difficult to regenerate from these. A total of 234 plants were eventually produced and 44% of these were spontaneous diploids. Attempts to microspore culture the backcross Mar34 x (Mar34 x GD33) were made but with no success (this was not unexpected as Mar34 is unresponsive to microspore culture). However, the other backcross GD33 x (Mar34 x GD33) has proved to be fairly responsive (giving anything up to 1600 embryos per 60 buds on one occasion). A total of 71 plants have been produced from this backcross; 50% of regenerated plants were spontaneous diploids. Both the DH lines and the BCDH lines were particularly difficult to seed. However, sufficient seed was produced for a final field trial of new DH lines in year 3. In addition an industrial partner carried out seed production of 119 DH lines as an in kind contribution.

37 DH lines from the Mar34 x GD33 cross together with the parental DH lines and cv Marathon were grown in the field at Warwick HRI. Harvested heads were assessed for post harvest performance in a shelf life room set up to mimic conditions in UK supermarkets (15°C and 24hr artificial lighting). 6 heads per replicate plot were assessed.
for shelf life. Temperatures in July and August 2006/07 were much higher than average with a maximum temperature of 36.5°C and some lines did not produce heads. The post-harvest quality of harvested heads was scored daily, assessing visual colour and stem turgor using a previously developed three point scale for acceptability Where possible head colour was also measured quantitatively using a Minolta CM-503i spectrophotometer, however, some lines developed atypical heads in the field – uneven or loose with purple or yellow patches and interposing leaves and it was impossible to take sensible Minolta colour readings of these, so they were only assessed visually.

The broccoli linkage map data generated by a previous Ph.D. student was checked for quality and re-mapped. Nine linkage groups were confirmed. Tissue and DNA samples were secured from the original and additional DH lines as they were produced and the mapping population on which the Mar34 x GD33 linkage map is based upon was increased to 154 DH lines; the map now consists of 28 SSRs & 106 AFLP loci and covers 946.7 cM. The map is currently being anchored to published linkage maps for B. oleracea QTL analysis of data for days to yellowing identified 2 additional QTL on linkage group 1 and linkage group 7 which accounted for 38% and 185 of the genetic variation respectively.

Conclusions and future work: The objective was achieved. The QTL analysis was completed by increasing the mapping population to 154 DH lines and increasing the number of DH lines for which phenotype data was available. The genotype and phenotype data and the mapping population was released to a BBSRC funded Industrial partnership award (Mapping and analysis of genetic loci controlling quality traits in broccoli) involving Syngenta Seeds and future work on a genetic approach to extending shelf life and post harvest nutrient quality of broccoli will be done within this project and also a BBSRC Crop Science Initiative studentship (Investigating the Post-harvest Genetic Control of Shelf Life and Metabolite Content in Broccoli). Both of these projects are providing the underpinning knowledge and tools required for a genetic approach to improving the post harvest quality of broccoli. including its nutrient quality.

13. Release breeding lines with leek rust resistance to breeding companies

Introduction: Work in HH0914 successfully developed leek breeding material with high levels of resistance to leek rust (Puccinia porri) derived from the wild species Allium commutatum. This material was potentially of value to commercial leek breeders and the aim of the work in this project was to carry out a 'technology transfer' exercise to demonstrate the merits of the material to breeding companies and to release a ‘leek rust resistance package’ to commercial breeders consisting of:
   - Appropriate resistant germplasm
   - Information to facilitate its exploitation.

Method and results: Leek plants selected from a ‘leek rust’ field trial carried out in 2003/04 previous to the start of this project were lifted and seeded in a glass house over the summer of 2004. The amount of seed produced varied between a few seeds up to 6 or 7 grams, however, sufficient plants seeded well enough for inclusion in a demonstration trial in 2005/06.

The 2004/5 ‘leek rust’ field trial consisted of 30 lines including 10 lines previously grown in the 2003/4 trial for confirmation of resistance, 12 lines previously tested for leek rust resistance in a glasshouse test for comparison with field performance plus parental lines and other controls and some additional observation plots. The plants grew well and the disease spread throughout the trial was very good providing a good robust test which confirmed the resistance in the breeding lines. The trial was assessed for rust resistance on a 1 – 9 scale in late April 2005. The top ten most resistant lines ranked almost exactly the same as in the previous trial and there was good agreement between resistance identified in glasshouse tests and the field. Morphologically the most resistant lines still possessed some characteristics of the Allium commutatum source of resistance but their level of resistance made them potentially useful breeding material for seed companies.

A demonstration trial was grown during 2005/06. The trial included 26 F4 and 6 F3 leek × A. commutatum lines plus the most resistant F2 family and Wintra (leek parent). Six commercial varieties donated by seed companies were also included to provide a ‘baseline’ of currently available germplasm. The trial also incorporated a small demonstration of disease resistance, a trial of 8 lines – 1 F3, 4 F4s, 1 susceptible F2, A. commutatum, Wintra and the F1 Kenton a partially resistant commercial variety from Nunhems. Representatives of nine seed companies (Semenis, Rijk Zwaan, Elsoms/Bejo, Syngenta, Nickersons, Nunhems, Tozer and Enza Zaden) viewed the trial. The breeding lines showed very good resistance to rust. Agreement was eventually reached with Nunhems Zaden and 114 breeding lines have been released to them.

Conclusions and future work: The objective was achieved and leek rust resistant breeding material was released to the world market leader in commercial leek breeding. Future breeding work for rust resistance will now be carried out as a commercial breeding activity by Nunhems. At present there are no plans for continuing leek crop improvement work at Warwick HRI

14. Assemble an onion diversity foundation set

Introduction: Onion (Allium cepa) is an obligate outbreeding species; there are therefore the same difficulties associated with screening heterogeneous populations as for vegetable brassicas. A more defined and structured set of genotypes representing the variability found in the Warwick HRI GRU collection would provide a basis for crop improvement research in onion and progress the identification of useful genes in a similar way to the Brassica DFFSSs. However, onion suffers from such severe inbreeding depression and it is only feasible to carry out one or possibly two generations of inbreeding and still maintain an acceptable level of viability. Although it is
15. Develop a methodology to identify novel sources of resistance to onion downy mildew
With Defra’s agreement no work was carried out on objective 15 and this was substituted by additional effort to achieve objective 17.

16. Generate a generic resource for lettuce crop improvement

Introduction: Previous Defra-funded research at HRI (HH0911SFV) on lettuce crop improvement resulted in the development of a mapping population of 342 F6 recombinant inbred lines (RILs) of lettuce derived from the cross Saladin x Iceberg (SalxIce) and a linkage map based on the F5 parental plants of the RILs and an F2 population. Many other ‘lettuce’ linkage maps are based upon mapping populations derived from interspecific crosses between lettuce (Lactuca sativa) and a wild species relative such as L. serriola or L. saligna; much of the polymorphism observed in such populations has been ‘bred out’ of the cultivated crop. The SalxIce RILs and associated map are therefore of very direct relevance to lettuce crop improvement research and they form the basis of collaborations with several breeding companies and other research groups. The parents of the mapping population also show significant variation for several important traits. The RILs and linkage map can therefore be used to carry out a genetic analysis of these traits. However, in order to improve the efficiency and effectiveness of such analyses there is a need to develop further both the mapping population and the associated linkage map information as a generic publicly available resource for lettuce crop improvement research.

Methods and Results: The ‘genetically’ most informative RILs in the SalxIce mapping population were identified by analysing the genotype data (available as of Feb/Mar 2004) using the ‘Genoplayer’ software from UC Davis. Lines were ranked according to the number of recombinations across all the mapped linkage groups. From this ranking, a set of 130 lines with the most recombinations was chosen. These were the genetically most informative lines; together they include 73% of the mapped recombinations in the mapping population. Lines were ranked according to the number of recombinations across all the mapped linkage groups. From this ranking, a set of 130 lines with the most recombinations was chosen. These were the genetically most informative lines; together they include 73% of the mapped recombinations in the mapping population.

In order to produce F7 seed of this core set of RILs, a single F6 plant from each of the 130 selected most informative RILs were grown to seed in an insect proof screen house. Reasonable to good amounts of seed were produced from 124 lines and this was catalogued and archived in the W HRI genomics resources centre for use in future studies. All F7 lines were then gowned for leaf samples and DNA extraction; the DNA together with leaf material for each line was archived in the Warwick HRI genomic freezer archive facility.

An additional F2 Lactuca serriola x cv. Iceberg mapping population was produced. The L. serriola parent is also a parent of the UC Davis mapping population and the F2 was used to consolidate the SalxIce linkage map through common markers. Through collaboration with UC Davis and two commercial lettuce breeding companies we have added more markers to the SalxIce linkage map which now consists of 276 loci and has a total map length of 626 cM with a mean marker distance 3 cM. 17 polymorphic EST sequences were identified for mapping
from a total of 40 potential markers screened. The map has been consolidated to 13 linkage groups; 8 of these groups have been positively anchored to other public or commercial lettuce maps and a further 4 groups tentatively anchored (1 common marker only). The map is due to be published shortly (9), after which it will be made available on the new VeGIN website.

Conclusions and future work: The objective has been achieved, although map improvement is an ongoing activity and will continue in the future. The SalxIce population and associated linkage map are valuable generic resources for lettuce crop improvement and are the basis of funded collaborations with several lettuce breeding companies, a BBSRC Crop Science Initiative CASE award on lettuce post harvest discoloration with a breeding company and a collaborative BBSRC funded project on attachment of human pathogens to salad leaves as well as trait based research in this project.

17. Complete a genetic analysis of nitrate content of lettuce

Introduction: Concerns about high nitrate levels in some vegetables led to the imposition of EU limits on nitrate concentration in lettuce (10). These limits raise problems for growers in northern European countries, such as the UK, because poor light quality can restrict the energy available to reduce and assimilate nitrate taken up by crops grown under protection. The nitrate content of a crop is a product of the nutritional environment and the crop genotype. Previously we have demonstrated that the parents of the SalxIce lettuce mapping population differ significantly for nitrate accumulation and have shown that this variation is representative of what can be found in a small sample of 24 lettuce lines.

Previous research on the genetics of nitrate content in lettuce demonstrated that this was a quantitative trait (11 & 12). Although the heritability of nitrate content appeared to be high there are many environmental factors which influence nitrate content making phenotypic selection difficult (13). The demonstration of significant variation between the parental genotypes for nitrate accumulation provided an opportunity for new research to characterise the genetic basis of this trait in lettuce and to provide lettuce breeders with the tools to manipulate this trait.

Methods and results: In order to assess the genetic variation for nitrate accumulation in the lettuce gene pool a lettuce Diversity Set (DS) was constructed comprising 45 different lettuce (Lactuca sativa) accessions and 3 accessions of the wild relative (L. serriola). The DS lines were selected from accessions held in the Warwick HRI GRU, breeding lines supplied by lettuce breeding companies and a number of summer and winter commercial cultivars currently used by UK protected lettuce growers. The DS therefore represented ‘old genetics’ (including undomesticated genotypes) and the genetics available to growers today. For the QTL analysis for nitrate accumulation 96 F7 SalxIce RILs were phenotyped for nitrate accumulation. Both the diversity set and the RILs were grown as summer crops (harvested between April and September) and three as winter crops (harvested between October and March), as defined by European Law (10). Plants were raised as transplants and then grown on either in solution culture in a glasshouse using the Nutrient Film Technique (NFT) with commercial rates of nutrient supply or in soil under a Spanish tunnel using a conventional fertiliser. All plants were harvested on the same date at, or slightly before commercial maturity of the faster growing lines in each experiment to minimise possible effects from variations in light; and the fresh and dry weight of the lettuce heads (whole shoots without roots) recorded. Nitrate concentrations in the heads were determined on sub-samples of dried and milled material and the results expressed in two ways according to convention; as an elemental concentration on a dry weight basis (% Nitrate N, ie g N per 100g DM) to allow comparisons with other elemental forms of plant N or converted to a fresh weight basis (mg nitrate per kg FM, ie ppm) using measurements of shoot water contents; this permitted comparisons with EC limits for nitrate concentrations in lettuce to be made. The % Organic N (ie reduced forms of N) and % Total C concentrations in the head dry matter were also measured.

There was significant genetic variation in nitrate concentration in the Diversity Set under both winter and summer conditions. Seasonal differences in nitrate concentration were relatively unimportant compared with differences between lines, partly because pre-harvest differences in light levels between the summer and winter experiments were smaller than expected, and partly because nitrate supply to plants grown in solution culture remains high throughout growth. The ranking of lines varied depending on whether nitrate concentration was expressed on a dry or fresh weight basis, due to varietal differences in shoot water content. This was particularly noticeable for the three L. serriola lines, which accumulated the lowest concentrations in shoot dry matter, but were ranked much higher when concentrations were expressed on a fresh weight basis because of their relatively low water content. The summer types had a lower minimum and higher maximum nitrate concentration (ie a larger range) than the winter types, although the mean concentration was slightly higher for the winter types. The mean N concentration was greater for modern genotypes than for the older ones, with a greater proportion of lower nitrate varieties in the latter group (Table 9). The range of concentrations was also smaller for the newer varieties suggesting selection of more recent varieties has not taken reduced nitrate accumulation into account. Saladin and iceberg (parents of the SalxIce Population) accumulated nitrate to significantly different extents, with concentrations lower in Saladin irrespective of how they were expressed. This indicated the SalxIce population was suitable to use for a genetic analysis of the trait.

There was significant variation in nitrate concentration between the RILs, although this was less than found in the diversity set. QTL analysis of the ‘summer’ and ‘winter’ data separately identified 13 QTL (Fig 7) in total affecting N accumulation in lettuce; 14 QTL for accumulation of N under ‘summer conditions’ and 12 QTL accumulation of N under ‘winter conditions’. Some of these collocated and 2 QTL appeared to affect N accumulation in both growing seasons. There was also overlap of 2 QTL affecting nitrogen on a fresh weight or dry weight basis under ‘summer conditions’ so that overall it appears we have identified 10 QTL for N accumulation in lettuce.
Table 9: Summary statistics for Nitrate accumulation in accession of the lettuce diversity set

<table>
<thead>
<tr>
<th>Statistic</th>
<th>% Nitrate N Concentrations</th>
<th>Nitrate (ppm FM) Concentrations</th>
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</thead>
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<td></td>
<td>Winter old</td>
<td>new</td>
</tr>
<tr>
<td>Age</td>
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<td>22</td>
</tr>
<tr>
<td>No of cvs</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
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<tr>
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</tr>
<tr>
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<td>-1.568</td>
</tr>
</tbody>
</table>

Figure 7. Position of QTL for nitrate accumulation on SalxIc linkage groups
However, this does not take account of the possible involvement of other factors such as water content in nitrate accumulation. QTL analysis of water content from the glasshouse experiments identified a significant QTL on LG3 which overlapped with a nitrate content QTL in the winter experiments but not in the summer. The results from the experiment in soil illustrate this complexity, as no significant QTL were found for nitrate content, but additional overlapping QTL for water content and organic Nitrogen content were found on linkage group c2 that were not significant in the NFT experiments.

**Organic N**
The amounts of Nitrate N and Organic N in the lettuce heads increased with head size (both fresh weight and dry weight) in all of experiments. The amount of Nitrate N were more strongly correlated with shoot fresh weight reflecting the strong association of nitrate with plant water, whereas Organic N was more strongly correlated with the weight of dry material, of which it forms a significant component. However, the concentrations of both forms of N in the plant were virtually independent of each other, indicating that Nitrate N did not accumulate at the expense of organic forms of N

**Water Content**
The concentration of Nitrate N increased with the water content of the heads (and decreased with their dry matter content) in all lines and all experiments. This emphasises the role of plant water in nitrate accumulation, and explains why differences in ranking of varieties occur when nitrate concentration is converted from a fresh to a dry weight basis. The relationships between nitrate concentration and plant water content is also consistent with independent measurements on lettuce grown under different light and/or N supply conditions, which show that volume regulation of plant cells is likely to be an integral part of an iso-osmotic mechanism for the control of nitrate accumulation in lettuce (14).

**Plant weight.**
Although the effects of plant size on nitrate concentrations in the Diversity Set were not significant, largely due to the greater variability between varieties, there was a tendency for nitrate concentrations to decrease with plant weight across the RILs, particularly under summer conditions $(P<0.001)$. Again this suggests the need for a QTL analysis for plant weight to determine if any of the N accumulation QTL co-locate. This also suggests that breeding for smaller or more compact heads may increase the risk of nitrate accumulation.

**Conclusions and future work:** The objective was achieved and 13 QTL for nitrate accumulation in lettuce have been identified. Several of these QTL co-locate on the genetic map, strongly suggesting a role in controlling nitrate level. However, we have no knowledge of the mechanism underlying the QTL or whether they are associated with nitrate metabolism, transport or accumulation. This limits their exploitation in a breeding programme. In future work we will initiate work to ‘dissect’ the complex trait of nitrate level to better inform breeding programmes aimed at improving the ‘nitrate economy’ of the lettuce crop. This will involve more detailed QTL analysis of traits that appear to influence nitrate accumulation in lettuce such as water content and plant weight to determine if any QTL co-locate and mapping polymorphic ESTs of genes associated with nitrogen metabolism in lettuce mined from the UCDavis EST database (http://compgenomics.ucdavis.edu/compositae_overview.php). Once the SalxIce linkage map is anchored to the integrated lettuce map additional SNPs in the region of the QTL and map any that are polymorphic between Saladin and Iceberg can be identified and used to redefine the QTL. Because the SNPs are derived from ESTs it will be possible to determine the gene function of SNPs mapping to the QTL. This information together with the mapping of ESTs associated with Nitrate metabolism/transport/accumulation will be used to identify potential target genes for other approaches such as TILLING and/or a gene sequence diversity approach using an expanded lettuce diversity set.

### 18. Complete a genetic analysis of Field Resistance to lettuce downy mildew

**Introduction:** Resistance to *B. lactucae* is a major target in all lettuce breeding programmes worldwide. However, lettuce breeders have almost exclusively utilised major (Dm) gene resistance deployed as single genes and since there is a gene-for-genec relationship between lettuce and *B. lactucae* (15) the resistance “breaks down”. An alternative form of host resistance is therefore required. The cv Iceberg has a high level of quantitative FR which is apparently effective against all pathotypes of *B. lactucae* and since the cultivar has been grown since the mid 19th century (16) the resistance appears to be durable. However, it has not been successfully utilised by lettuce breeders mainly because of the difficulties of accumulating sufficient alleles for resistance while at the same time maintaining quality characteristics. Markers for the loci determining resistance will allow marker assisted selection for resistance to be carried out in the absence of environmental influences. Previously we identified 3 QTLs determining downy mildew disease levels at harvest (HH0911SFV), however, we do not know what components of FR are affected by the different QTLs. In this project the aim was to develop an assay which allows us to measure different components of quantitative resistance (latent period, degree of sporulation, spore viability etc) and carry out a dissection of the gross phenotype (amount of disease at harvest).

**Methods and Results:** Improvement to the SalxIce linkage map (see section 16 above) has resulted in refinement of the QTL for disease levels at maturity.

A number of lab based tests for different possible components of field resistance to *Bremia lactucae* were developed using 12 genotypes of lettuce with known different levels of field resistance to *B. lactucae*. These included a leaf disc test and leaf strip tests to assess latent period, spore germination on lettuce leaves and spore vigour as measured by % germination on agar and germ tube length in liquid culture. A ‘Matlab’ image analysis programme was written to analyse % germination and germ tube length. All tests gave interesting results with a degree of correlation with results from the field. The best agreement with the field data was obtained by
assessment of latent period and it was decided to use this assay to obtain data from the most informative RILs in order to attempt to assign components of resistance to previously identified QTLs for field resistance to *B. lactucae*. Using genotype information a subset of RILs containing different Dm QTL combinations was identified for phenotyping.

*Bremia lactucae* isolate B10/96 was selected for the experiments as this isolate reportedly possesses the matching virulence factors for the Dm genes segregating in the Salxlce RILs. Two experiments were done initially to confirm the isolate’s resistance profile; cotyledons of seedlings of a set of differential lines possessing different resistance (Dm) genes were inoculated with a spore suspension of B10/96. The results indicated that the isolate possessed the matching virulences for all of the known Dm genes segregating in the Salxlce RILs. Three leaf strip experiments were then carried out to optimise the methodology and establish a working protocol to enable further high throughput testing of the RILs. The method for each experiment was based on a previously defined method and was done on a limited selection of lettuce lines which were tested blind. The results of these initial experiments were consistent with one another; inoculating the abaxial (lower) surface resulted in faster sporulation than inoculation of the adaxial (upper) surface of the leaf. The test distinguished between the resistant (Iceberg) and susceptible (Saladin) parents consistently and also identified RILs which had previously been shown to be resistant and susceptible in the field. The protocol was then used to assess a larger number of RILs for their latent period.

A hundred of the Salxlce F7 RILs were selected and tested. Saladin and Iceberg were included in each experiment as positive and negative controls along with the F6 RIL LJ00092 which was shown to possess a high level of ‘field’ resistance in previous studies. Each line was tested 3 times following a randomised plan and assessed for the presence or absence of sporulation at 4, 5, 6, 7.8 and 11 days after inoculation. Eleven lines did not show any sporulation after 11 days; only 2 of these were among the most resistant lines in previous field experiments. QTL analysis for the mean time to first sporulation identified one putative QTL on linkage group 10 with a LOD score of 2.54; this QTL was new and independent of the QTL for field resistance identified previously.

**Conclusion and future work:** The objective was achieved; an additional QTL for a defined component of field resistance to *B. lactucae* (latent period) was identified using a newly developed lab based test. The data from this project have been used in a parallel collaborative with a commercial lettuce breeding company on developing a strategy for breeding for QTL based FR to *B. lactucae*. This work is ongoing and there is a need to fine map the QTL for resistance in order to identify tighter markers. Ultimately it would be of great interest to identify the genes underlying the QTL for FR as this would provide insight into the mechanism of this type of resistance and also possible reasons for its durability.

**Knowledge transfer**

From the above report it can be seen that the project has successfully carried out its purpose to develop a range of underpinning resources and information about genetic variability for specific traits of interest, in a form suitable for use in crop improvement research in vegetable brassicas, lettuce and onion. The original proposal stated that “The route of delivery for this research to growers will largely be through technology transfer to commercial plant breeding companies.” The project has effectively operated as a network and had significant interaction with industry and other researchers. The technology transfer to industry of plant material and information from this project is summarised in fig 8.

In addition the project has received support ‘in kind’ from industry, namely

- Regeneration of lettuce RILs
- Assistance in microspore culture for Bol DFFS production
- Regeneration of the AG mapping population
- Regeneration of the Mar34GD33 mapping population
- Lettuce varieties for Nitrate work
- Markers for lettuce linkage map

Other ‘industry related’ KT outputs from the project include:

- 6 KT articles in the trade press
- 17 KT presentations to grower and breeding company representatives
The project has also delivered plant material and information to other research projects (Fig 9). Overall the project has been instrumental in generating approximately £3m of government and industry funding.

Figure 9: Knowledge transfer - outputs to other projects

References:
9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

**Referred Publications:**


Other scientific publications:


Knowledge transfer

KT articles

Pink D A C & Teakle G (2005) Bringing old genes into modern crops. The Vegetable Farmer, February, 2005


KT Presentations


Pink D A C. Defra funded crop improvement research. HDC Field Vegetable Panel meeting 8 March 2006

Pink D. A. C Crop Improvement of Field Vegetables HH3723, WHRI Kirton KT event , 28 June 2006

Pink D A C. Crop Improvement at Warwick HRI Talk to ADAS Workshop 22 Sept 2006

Pink D A C. Trait based Brassica breeding research at Warwick HRI, Presentation to Brassica group of Plantum (Dutch plant breeding organisation) September 2008.

Pink D A C. An “overview” of lettuce breeding research at Warwick HRI. Presentation to British Leafy Salad Growers conference, 19 Nov 2008

Pink D A C Improving broccoli shelf life – presentation to Brassica Growers Association AGM, Jan 2009

Roberts, S.J., Vicente, J.G. & Everett, B. 2005 - Comparison of pathogenicity and PCR tests for confirmation of Xanthomonas hortorum pv. carotae. 5th ISTA Seed Health Symposium, Angers, France, 10-13 May 2005.


iCASTS and other media


Biofuels from brassica – describing brassica diversity sets
http://www2.warwick.ac.uk/newsandevents/icast/archive/s2week1/brassicas/

Building better broccoli – describing shelf life work in broccoli
http://www2.warwick.ac.uk/newsandevents/icast/archive/week17/broc/

Information on the current status of the brassica diversity sets
http://www.brassica.info/resource/plants/diversity_sets.php

D Pink was interviewed for Radio 4 6 o’clock News, Farming Today and The Today programme and local radio about work on broccoli shelf life.

D Pink was interviewed by Sheila Dillon for the Food programme about using wild brassica species in vegetable breeding.

Other KT activities

Information regarding Xanthomonas and Peronospora differentials has been passed to two seed companies.

Three confidential commercial contracts are on going with seed companies based on work carried out in previous Defra projects and this project.

One BBSRC Case award with Rijk Zwaan is based on information and resources generated in HH3723

Three BBSRC industrial partnership award projects with Syngenta and a BBSRC dietary Research Industry Club project are based on resources generated in HH3723.

A KTP project with Elsoms Seeds Ltd based on knowledge generated in HH3723