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SID 5 [Research Project Final Report]

- **Note**
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### Project identification

1. **Defra Project code**  
   IF0125

2. **Project title**  
   OREGIN: Oilseed Rape Genetic Improvement Network to deliver strategic objectives for Sustainable Agriculture and Climate Change

3. **Contractor organisation(s)**  
   Rothamsted Research
   University of Warwick

4. **Total Defra project costs**  
   (£ 250,000)

5. **Project**  
   start date: 01 April 2007
   end date: 31 March 2008
Executive Summary

7. 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 first objective was to demonstrate a practical approach for identifying and utilising available genetic variation relating to oilseed rape (OSR) crop traits that address and assist in the delivery of strategic objectives for environmentally sustainable farming, by carrying out time bound pilot experiments on prioritised traits. We demonstrated that there was considerable variation within diversity sets established by the OREGIN project for the ability to take up and utilise nitrogen fertiliser. Moreover, there were differential growth responses to high and low nitrogen supplies, suggesting that the experimental approaches and materials used may guide selection of genetic material optimised for low N input production systems. Investigation of a key gene associated with nitrogen utilisation indicated that many copies are present within the oilseed rape genome, and that this needs to be taken into account for future molecular genetic studies. For a gene associated with oil quality we demonstrated the ability to screen for and identify novel genetic variation in mutagenised populations. We demonstrated that there was considerable variation in oil and protein content and protein composition within the diversity sets, and that these traits were amenable to genetic analysis and resolution. This will underpin future efforts to optimise seed composition in relation to efficient use of input resources. We demonstrated the value of using specific immunological detection of seed proteins to select material with modified protein composition.

The second objective was to ensure that integrated datasets and information describing specific crop traits, relevant genetic variation and reference resources in OSR are available in a form that is readily usable by the research and stakeholder communities, and can assist in prioritising traits for crop breeding. Work was completed on refining and harmonising the CropStore database structures, and standardising methods of data acquisition and curation. This will improve the ability of researchers and breeders to collectively assess the ability to identify relevant genetic variation and determine the likely downstream work involved in detailed genetic characterisation and resolution prior to exploitation in commercial breeding programmes. Datasets associated with the plant genetic resources developed and distributed by OREGIN have been identified, collated and curated, and are now available for querying and delivery from the CropStoreDB database. Data are now curated to a high quality, providing long-term added value for geneticists/breeders. Data summaries and detailed datasets are delivered via a simple web interface and will allow direct comparison of accumulated datasets in future. Datasets associated with the resources developed within the OREGIN project have been checked and collated in a consistent format. A method has been developed for making these data readily available in the public domain. Data have been made available via the OREGIN and www.brassica.info websites.

The third objective was to provide the OREGIN experimental resources (DFFS sets representing plant genetic diversity, reference mapping populations for genetic resolution, and pathogen collections) to the OSR R&D and breeding community, together with associated information to enable rapid genetic analysis of sustainability traits. Progress has been made in this area, with decisions on future strategy for fixing lines. Molecular marker surveys have been extended to provide information on genetic diversity within the experimental material managed and distributed by OREGIN. Larger scale bulking of genetic mapping populations has been carried out. Single spore isolate sets of major UK fungal pathogens have been generated, with associated molecular markers providing information on genetic diversity. Assessments of disease incidence amongst diverse genetic material have been made and analysed, and standard
operating procedures made available via the OREGIN website.

The final objective was to provide a basis for environmentally sustainable agriculture in relation to predicted climate change by characterising the OREGIN biological resources in the context of adaptations to climate change and modelling predicted effects of climate change to inform future policy, breeding and farming decisions. The model predicted that warmer winters would significantly advance the date of stem canker appearance in spring and increase the severity of canker before harvest. The model also predicted that epidemics will spread north from England to Scotland, where canker does not currently occur on oilseed rape.

In conjunction with the OREGIN management committee and other stakeholders, it has been agreed that future work should concentrate on delivering a breeders' and pre-breeding 'toolkit'. This requires i) genetic markers in the context of reference linkage maps; ii) a defined range of germplasm representing significant, useful allelic variation; iii) information to place these resources in context of trait variation amenable to breeding selection. This will be achieved by 1. maintaining and developing OREGIN seed and pathogen genetic resources, to enable resolution and introgression of useful variation; 2. by quantifying and characterising genetic variation for key traits; and 3. delivering the pre-breeding toolkit through provision of integrated information to maximise utilisation of genetic diversity. Particular emphasis will need to be placed on data collation and integration, as this underpins all relevant activities by researchers, breeders and associated LINK consortia.

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;
- the main implications of the findings;
- possible future work; and
- any action resulting from the research (e.g. IP, Knowledge Transfer).

Scientific Objectives

1. Demonstrate a practical approach for identifying and utilising available genetic variation relating to crop traits that address and assist in the delivery of strategic objectives for environmentally sustainable farming, by carrying out time bound pilot experiments on prioritised traits.

2. Ensure that integrated datasets and information describing specific crop traits, relevant genetic variation and reference resources in OSR are available in a form that is readily usable by the research and stakeholder communities, and can assist in prioritising traits for crop breeding.

3. Provide the OREGIN experimental resources (DFFS sets representing plant genetic diversity, reference mapping populations for genetic resolution, and pathogen collections) to the OSR R&D and breeding community, together with associated information to enable rapid genetic analysis of sustainability traits.

4. To provide a basis for environmentally sustainable agriculture in relation to predicted climate change by characterising the OREGIN biological resources in the context of adaptations to climate change and modelling predicted effects of climate change to inform future policy, breeding and farming decisions.

Report on Work Programme

Work Package 1. Demonstrate a practical approach for identifying and utilising available genetic variation relating to crop traits that address and assist in the delivery of strategic objectives for environmentally sustainable farming, by doing time bound pilot experiments on prioritised traits
Fertiliser N is taken up through the roots, assimilated into organic compounds, transported to where it is required in the crop plant and often subsequently remobilised to new locations, especially during seed development. Thus Nitrogen Use Efficiency (NUE) is a complex trait where many steps, or component traits, can contribute to the overall NUE. We took three approaches to provide underpinning information on the genetic potential for improvement of NUE in OSR.

1.1 VARIATION OF OSR NITROGEN ASSIMILATION

1.1A DIVERSITY ANALYSIS

Methodology

The Defra-LINK project (LK0979 "Breeding oilseed rape with a reduced requirement for nitrogen fertiliser", hereafter referred to as the NUE-LINK project) involves developing techniques to identify NUE component traits, and characterisation of a restricted range of winter OSR germplasm in the field under varying levels of N fertiliser. Future work will involve screening OSR genetic mapping populations in the field and glasshouse, in order to identify segments of the genome (quantitative trait loci or QTL) that are responsible for controlling NUE-related traits.

We previously demonstrated, using SSR genetic marker analysis of the OREGIN *Brassica napus* Diversity Foundation Set (BnaDFS), that *B. napus* crop types represent distinct gene pools. Here we tested the hypothesis that the BnaDFS is likely to be a useful source of genetic variation for NUE component traits which may exceed that present in the lines used in the NUE-LINK project. The number of lines being screened in the NUE-LINK project is limited by the cost of performing replicated field-scale trials and the practical requirement to grow lines of the same crop type to enable meaningful comparisons to be made.

In order to obtain information on genetic variation for a wider sample of the gene pool representing more diverse lines, we carried out a pilot scale glasshouse-based screen using the nutrient film technique (NFT) hydroponics facilities at WHRI. 103 lines were screened at high and low N concentrations (8 mM and 2 mM, respectively) in a fully randomised replicated design for 8 weeks, prior to floral development. The set included 92 lines from the BnaDFS, 9 lines from the NUE-LINK project and 3 from the TNDH mapping population. This allowed additional comparisons to be made, including the relationship between field and glasshouse experiments. All aerial tissue was harvested, fresh and dry weight recorded, and samples subjected to mineral analysis. This included N content incorporated into organic compounds (organic N), mineral nitrate (NO₃), sodium (Na), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg) and manganese (Mn).

Results and Analysis

The results demonstrated a considerable variation in growth and mineral uptake across the lines at the two N concentrations. Fresh weights were very closely correlated with dry weights. At high N we observed a four-fold range in average dry weight accumulated between the fastest and slowest growing lines, while at the low N supply there dry weight varied over a 3.4-fold range (see Figure 1).

![Figure 1. Ranked average dry weight at low N. 92 lines from the BnaDFS, 9 lines from the NUE LINK project and 3 from the TNDH mapping population were grown for 8 weeks in a randomised replicated design using the NFT facilities at WHRI, at either 8 mM (high) or 2 mM (low) N supply. The chart shows the average dry weight of total aerial tissue for each line from the low N treatment sorted in rank order. Lines marked with black circles are from the NUE-LINK project.](image)

Although, as expected, the average dry weight at high N was higher than that at low N, the ranges overlapped by 77%. This indicates that many lines grew faster at low N than others did at high N. In addition, we found variation amongst the lines in terms of growth response to N level. Some lines responded well to increased N, and others were non-responsive. Tapidor DH was the slowest grower at High N and one of the slowest at Low N. TapidorDH is derived from the variety Tapidor and is the female parent of the TNDH mapping population. It was also consistently the poorest yielding line in the NUE-LINK project field trials last year. The remaining 8 lines associated with the NUE-LINK project cover about half the range of values of the BnaDFS at high N, and are mostly clustered around the central values at low N (marked by black circles in Figure 1). The exception to this was the line FDS02, which produced the highest biomass of all the lines at each treatment.

Nitrate and organic N together comprise most of the N that has been taken up by the plants, since ammonium is maintained at low concentrations. Nitrate levels can fluctuate within a plant depending on the light
intensity driving photosynthesis. However, we observed a 1.6 (at high N) and 1.5-fold (at low N) variation in nitrate concentrations between lines. A similar range of organic N concentrations was also observed, with 1.49-fold variation (2.5 - 3.7% of dry weight) at high N and 1.55-fold variation (2.7 - 4.2% of dry weight) at low N.

Although this variation appears to be over a relatively narrow range, it does indicate the presence of genetic variation within the genepool. However, when the amount of plant growth is taken into account, a greater range (~4-fold) of total N assimilation is apparent (Figure 2). Again, line FD502 performed best, with essentially the same levels of assimilation under both high and low N. In agreement with the results from the NUE-LINK project, TapidorDH was amongst the poorest performers. The remaining 7 lines from the NUE-LINK project were distributed around the central values, similar to their distribution with respect to dry weight data. The three TNDH lines had a distinct range of values, as did their dry weights.

Figure 2. Comparison of organic N assimilation at high and low N. 92 lines from the BnaDFS, 9 lines from the NUE LINK project and 3 from the TNDH mapping population were grown for 8 weeks in a randomised replicated design using the NFT facilities at WHRI, at either 8 mM (high) or 2 mM (low) N supply. The average total amount of N incorporated into organic compounds in the aerial tissues of each line is compared for the high and low N treatments.

Composition of six other elements (P, Mg, Ca, Na, K, Mn) was observed across a range of 1.36-fold to 2.05-fold variation (Table 1). The ranges for Ca and Mg are slightly less than the approximately two-fold variation observed in B. oleracea (Broadley et al., Plant Physiology, 2008), which may reflect the increased degree of genetic diversity in the diploid species.

Table 1. Observed range in mineral content variation in B. napus. Aerial tissue was harvested from 8 week-old plants of the 103 lines used in the NFT experiment, dried and assayed for the content of a range of minerals. The table summarises the fold-difference in mineral concentration between the highest and lowest values for each of the N treatments.

<table>
<thead>
<tr>
<th>Mineral</th>
<th>High N</th>
<th>Low N</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>1.64</td>
<td>1.54</td>
</tr>
<tr>
<td>Mg</td>
<td>1.74</td>
<td>2</td>
</tr>
<tr>
<td>Ca</td>
<td>1.76</td>
<td>1.88</td>
</tr>
<tr>
<td>Na</td>
<td>1.58</td>
<td>1.98</td>
</tr>
<tr>
<td>K</td>
<td>1.49</td>
<td>1.36</td>
</tr>
<tr>
<td>Mn</td>
<td>1.85</td>
<td>2.05</td>
</tr>
</tbody>
</table>

Regression analysis between each of the minerals assayed highlighted a number of significant correlations, the most striking of which was that between Ca and Mg at low N ($R^2 = 0.80$). This is in agreement with recently published data obtained from B. oleracea (Broadley et al., Plant Physiology, 2008).

Conclusions
As postulated, there does appear to be more genetic variation in the BnaDFS lines than in the NUE-LINK project lines included here. The BnaDFS contains the parents of several mapping populations, including those of the TNDH and DYDH populations. These two sets of parents show wide differences indicating that these populations will segregate for this trait, most likely with transgressive segregation. This is also supported by the large differences between the three TNDH lines sampled. These data will be valuable in aiding selection of a mapping population for use in the NUE-LINK project.

The data obtained in this study show genetic variation between lines for nitrate uptake, organic N assimilation, and differential growth responses to high and low N supplies. These can be considered component traits for NUE and are indicative that the BnaDFS will exhibit genetic variation for other NUE component traits.

A report on these results will shortly be made available on the OREGIN website, and will be presented to the NUE-LINK project participants, many of whom had already expressed interest whilst we analysed the preliminary results.

1.1B RELATIONSHIP BETWEEN N LEVEL AND SEED PROTEIN CONTENT
An additional pilot experiment was designed at RRES in order to determine whether it was possible to detect genetic variation in response of OSR to different N levels in relation to final seed protein content.
Methodology

In contrast to the NFT experiments at WHRI (section 1.1A), we sought to establish a system that would enable reproducible control of N level and sustain growth of plants to harvestable maturity. Plants were grown in a sand:perlite (50:50) mix under glasshouse conditions and provided with nutrient solution (as for the WHRI experiment) containing 2 mM or 8 mM nitrogen.

Results and conclusions

Initial results from reference Brassica lines indicate that there is considerable variation between genotypes in their response to N level, both in terms of plant height, and key yield indicators such as number of seed pods (siliques) produced, and silique length. Of most significance is the fact that some genotypes show little or no response to variation in N level, consistent with the WHRI experiment. Such observations may guide selection of genetic material optimised for low N input production systems.

1.2 Genetic variation of OSR glutamine synthetase

1.2A Natural variation in gene sequences

An alternative approach to screening germplasm directly for likely component traits associated with NUE is to characterise candidate genes (i.e. genes that are expected to have an important role). N is obtained from the soil largely as nitrate and ammonium and the first steps in the assimilation of nitrate involve the reduction to ammonium, which is also produced in large quantities during photorespiration and other plant processes. Ammonium is assimilated into glutamine, the first organic N compound, by the enzyme glutamine synthetase (GS; Habash & Miflin, 2002). Plants have evolved a family of GS isoforms, which in the closely-related model plant, Arabidopsis, is made up of five cytoplasmic (GS1) genes and one plastidic (GS2) gene, each of which has a distinct role during plant development. Previous studies suggest that altering GS activity can affect plant growth responses and yield, and that natural variation at GS loci may affect NUE. As a result GS could be a realistic target for selection in OSR.

Since the OSR amphidiploid B. napus genome is comprised of sets of up to 6 ancestral duplicated segments when compared to Arabidopsis, the GS gene family is expected to be correspondingly more complex. Our objective was to estimate the GS gene content of OSR, and obtain information on the allelic variation at selected loci.

Methodology

The proposed rationale for detecting allelic variation in GS loci relied on identifying sequence differences between the different GS genes to design primers that would be used to PCR amplify individual genes that could then be directly sequenced. This approach had successfully been applied to the sequencing of two fatty acid elongase (FAE1) loci from OSR within OREGIN (2006-7), and is desirable for sequencing many accessions as it does not require the more expensive and time consuming initial cloning step prior to sequencing. However, reliable design of gene-specific PCR primers requires sequence information on all the gene family members.

The initial approach was to search available Brassica sequence databases (including large EST datasets and data from the B. rapa genome project) for sequences homologous to the six previously published OSR GS sequences (Ochs et al., 1999).

Results and Analysis

We identified 225 sequence accessions, which were assembled into 56 variety-specific cDNA and genomic sequences, representing 25 A and C genome genes (summarised in Table 2). It was possible to assign 6 of the OSR genes to the A, and 9 to the C ancestral genomes. This suggests that there may be at least 15 GS genes within the OSR genome, although this is likely to be an underestimate due to incomplete representation in current sequence databases. It was possible to recognise 5 pairs of homoeologous loci between the A and C genomes. In contrast to many other plant species that have a single GS2 gene locus, and hexaploid wheat which has three, OSR appears to possess five loci. Contrary to our previous experience with other Brassica gene families, it was not possible to determine which Brassica GS1 loci corresponded to which of the five Arabidopsis GS1 genes on the basis of these sequence comparisons.
Multiple alignment of the 55 assemblies and the only published full-length genomic sequence allowed identification of most of the exon splice sites. We found that the locations of the 11 introns in GS1 and 12 in GS2 were highly conserved amongst all the distinct copies in *Brassica* and *Arabidopsis*, although two *Brassica* genes contained an additional intron.

The coding sequences were very highly conserved at the DNA level, although non-coding sequences of non-homoeologous loci were distinct (where genomic sequence was available). Moreover, there was only a 6% sequence divergence between particular homoeologous A and C genome GS2 loci within the 600 bp downstream from the transcription stop site - a region unlikely to be subject to high selection pressure for sequence conservation.

We designed and tested a range of primers located at polymorphic sites and used these in different combinations to carry out PCR prior to sequence analysis. With one GS2 primer pair, it was first necessary to subclone the PCR product prior to sequencing since mixed PCR products representing multiple loci were obtained from OSR. We also determined valuable additional genomic sequence for GS2 from *B. rapa* and GS1 from *B. oleracea*. The full sequence alignment appears in the report on the OREGIN website.

**Conclusion**

Due to the unexpected level of genome duplication at OSR GS gene loci, combined with relative high level of sequence conservation, we were unable to meet the original objectives of this task. Additional optimisation of the PCR reactions will be required to determine if the primers designed are gene-specific within OSR.

Although the design of gene-specific PCR reactions has been more difficult than originally anticipated, the sequences obtained have contributed valuable new genomic sequence data (two *B. oleracea* GS1 sequences corresponding to those within the OSR C genome are being published in Genbank: accession numbers EU822334 and EU22335). In addition, the GS gene alignments (on the OREGIN website) are a valuable resource for further characterisation of this gene family within the OREGIN network. A more detailed report summarising this work is available on the OREGIN website.

**1.2B TILLING SCREENING FOR NOVEL VARIATION.**

In order to assess the ability to identify novel induced variation at specific target loci such as GS in *B. napus*, we had planned to use the sequence information accumulated at WHRI to develop appropriate primers prior to screening an existing mutagenised population. This was to make use of the Targeted Induced Local Lesions IN Genomes (TILLING) approach and an EMS-mutagenised population developed by our colleagues at HAU, Wuhan, China. However, due to the complexities of the sequence analysis described above, and the lack of locus-specific primers available within the required time-span, we worked with colleagues at HAU to focus on a gene target that had previously been used within OREGIN to assess allelic variability within the diversity sets. Fatty acid elongase1 (*FAE1*) is the key gene in seed erucic acid biosynthesis in rapeseed, and had been mapped by Southern analysis to two loci within *B. napus*.

**Methodology**

Two ethylmethanesulfonate (EMS) mutant populations of the semi-winter rapeseed had been constructed from a spring rape variety (Ningyou7), one treated with 0.3% and one with 0.6% EMS mutagen. Paralogues of *FAE1* were characterised by screening a *B. napus* cv. TapidorDH BAC library. Distinct sequences from the BACs were used to identify marker polymorphisms which were used to determine map locations corresponding to functional loci affecting erucic acid content in the reference TNDH mapping population. A novel procedure was used to identify lines within the EMS-treated population that carried mutations within the *FAE1* loci. The procedure involved optimising PCR primers so that DNA samples from individual plants could be screened for sequence variants in situations where two or more very similar paralogous gene copies exist in a genome. The presence of known SNP (Single Nucleotide Polymorphism) variation between paralogous copies was valuable as an internal control for the TILLING fragment separation assay.
Results
The procedure enabled discovery of 19 FAE1 mutations from TILLING analysis of 1344 M2 plants, three of which were demonstrated to have reduced seed erucic acid content.

Conclusion
- The methodology developed in collaboration between RRES and HAU was a valuable exercise that makes it possible to target multiple sequence-conserved gene loci within the complex OSR genome, and identify functional mutations. This approach may also be relevant to Eco-TILLING of diversity collections.
- The screening with FAE1 demonstrated that the two B. napus mutant populations carry a high mutant load, and that this TILLING platform will be of considerable value for functional genomics in B. napus, as well as for introduction of novel allelic variation in rapeseed breeding.
- It should be possible to screen for mutant lines carrying novel sequence transitions in one or more of the GS loci described by WHRI, and use this as a basis to target functional variation within this population. Given the larger number of paralogous loci, it may be necessary to accumulate and combine a series of mutant alleles and match this to desired N use phenotype.
- A paper describing this work, and its application to targeted crop improvement, has been submitted for publication (New Phytologist).

1.3 Determination of Variation for Protein Content and Quality in OSR
Modulation of the rapeseed oil/protein ratio can contribute to achieving a higher and more valuable oil yield for any given production input. In order to generate datasets that provide information on which to base decisions about the extent, distribution and location of genetic variability within the Brassica genepool for oil/protein ratio, and protein quality, lines (the majority of seed were kindly provided by WHRI) representing the BnaDFS, reference OSR mapping populations and other Brassica species were assessed. An important aspect of this task was to determine the feasibility of large-scale screening of genetic resources and genetic mapping populations. The task was to determine variation in a) overall seed composition, and b) protein composition.

1.3A Seed Composition
Methodology
We assessed the alternative methods by which we could assess oil/protein ratios for genetic screening and analysis. Protein assessment by the Coomassie-dye based 'Bradford' assay is destructive and only accounts for soluble proteins. Nuclear Magnetic Resonance (NMR) is widely used but only provides information on oil content, and is sensitive to moisture content (Krygsman & Barrett, 2004). As a result, we made use of non-destructive NIRS (Near Infrared Reflectance Spectroscopy; NIRSystem 5000, Foss) analysis on whole seeds, using facilities kindly provided by the plant breeding company KWS-UK (formerly CPB-Twyfords). NIRS is used routinely by the seed and breeding industry and has been the method of choice for genetic screening to date (Zhao et al., 2008). In addition to quantification of seed oil and protein, the spectra obtained can be analysed to provide information on other traits such as glucosinolates and sulphur content. Two types of data were collected: raw spectra and calibrated data (referenced to a particular OSR variety) for oil, protein, glucosinolates, sulphur and moisture content. By using specific calibration equations, the raw spectra can be dissected further to obtain both amino acid (Fontaine et al., 2001) and fatty acid (Sato, 2008) content information, though this was not within the remit or timescale of this project.

Each sample was analysed in triplicate in randomised runs of 48 samples, with 2 controls per run to decrease machine/day variability. More than 1800 runs were performed. All calibrated data were represented as a linear mixed model analysed by REML (REsidual Maximum Likelihood), and estimates of heritability were obtained. The experimental design enabled us to identify and apportion sources of variation associated with line (heritability), accession, environment, sampling occasion, sample, batch and residual error.

In order to validate the NIRS results for oil content, and in cases where only small amounts of seed were available, we measured oil content using NMR instrumentation (MQC, Oxford Instruments) located at John Innes Centre, Norwich. The NMR required only ~200 mg seed for sampling, and so it was possible to select more uniform, high quality seed for analysis. More than 800 samples were analysed in triplicated randomised runs for the BnaDFS, and in a single run for the TVSL mapping population.

Results
Following analysis of the results we were able to compare the variation present within the segregating mapping populations (TNDH and TVSL) with that of the BnaDFS. Environmental effects were apparent, with seeds of the TNDH mapping population generated in different field trials being clearly distinguishable. The level of genotype x environment (G x E) interaction observed with the NIRS data indicates that care needs to be taken when assessing and ranking the range of diversity in material of different provenance. Overall, using the RRES-grown TNDH population as a baseline, we found that:
- The seed from the WHRI-grown TNDH population had higher protein, higher moisture, lower oil, and similar glucosinolates and sulphur.
• TVSL had lower protein with more variation, similar moisture, higher and more variable oil, and more variation in glucosinolates.
• Seed from the BnaDFS had slightly higher and more variable protein, higher moisture, lower and more variable oil, more variation in glucosinolates and sulphur.
• In comparison, *B. rapa* lines had slightly higher protein, similar moisture and oil, higher glucosinolates and sulphur.

A wide range of values were obtained (Figure 3): Within the BnaDFS, oil content measured by NIRS varied from 33.7-46.4% and protein from 19.2-28.8%. When assessed by NMR, oil content varied from 18.9-50.2%. Comparison of NIRS datasets with those generated from NMR indicated a surprisingly good agreement (Figure 5), for both the BnaDFS and the TVSL mapping population. The greater variability apparent in the BnaDFS data can be attributed to the increased variation in seed quality, seed coat colour and seed size compared to the TVSL population. Moreover, the standard calibration used in NIRS is based on particular OSR varieties and is unlikely to be appropriate for all samples within the BnaDFS, thus, some measurements may be skewed by factors not directly related to the target traits of seed oil or protein content. However, the raw spectra that we also collected could be adjusted with additional calibrations.

As expected, the relationship between seed oil and protein content was negatively correlated (Figure 4) and there was greater variation present within the BnaDFS than in the mapping populations.
Figure 5. Relationship between NIRS and NMR for %seed oil content in the BnaDFS and TVSL population.

Identifying genetic loci associated with seed traits
We collated existing published data relating to quantitative trait loci (QTL) responsible for variation in seed oil and protein content. These were then compared to the data generated from the seed NIRS and NMR analysis to identify significant QTL (LOD >3.0) in the context of the reference TNDH genetic linkage map (Qiu et al., 2006). This indicated that distinct loci regulated variation in seed oil, protein and glucosinolate content and in oil:protein ratio, with coincident QTL for glucosinolates and sulphur content on two homeologous chromosomes. The data for seed oil content were consistent with those previously reported for the TNDH population using material generated in China (Qiu et al., 2006; http://brassica.bbsrc.ac.uk/IMSORB/). Data were collated and placed on the OREGIN website.

Conclusions
From these experiments, we conclude that there is wide variation for seed oil and protein content in the BnaDFS and mapping populations. However, any future comparative work based on NIRS should ideally be based on careful generation of seed batches, in order to take into account variation due to Genotype x Environment interactions. Comparison of two or more seed batches for an overlapping range of genotypes generated in contrasting environments would provide a more accurate assessment of relative ranking across the gene pool. For some material (e.g. mapping populations) it would also be preferable to generate seed under consistent controlled environment conditions. We demonstrated the ability to locate QTL for oil and protein content which will underpin future efforts to select for these important traits in breeding programmes.

1.3B ANALYSIS OF SEED PROTEIN COMPOSITION
Rapeseed protein meal contains two predominant classes of seed storage proteins: 12S globulin (cruciferin) which represents 25-65% of its protein content and 2S albumin (napin) (Berot et al., 2005; Raab et al., 1992). Of these two components, napin has poorer quality in terms of amino acid composition and ingestion by livestock. Removal of napin from meal via conventional breeding is desirable and would provide new opportunities for meal markets through delivery of essential amino acids. Moreover, reducing napin content is expected to have a significant effect on the nitrogen economy, as there will be less wastage and recycling of nitrogen from animals. It should be feasible to considerably reduce or eliminate napin from breeding lines, without deleterious effect on seed quality, through genetic selection following identification of natural or induced variation that alters the ratio of cruciferin:napin.

Our task was to determine whether it was feasible to screen for genetic variation in the seed protein components within the Brassica gene pool and assess whether such variation could be assigned to specific genetic loci.

The molecular mass of cruciferin, a member of the 11S globulin family, is estimated to be ~300,000 Da in its native confirmation, with an isoelectric point (pI) of ~7.2. At extreme pH and in urea solutions, the protein totally dissociates into six subunits, each comprising two polypeptide chains (alpha and beta) of ~ 30,000 Da and 20,000 Da linked by a disulphide bond (see Figure 6). Three major groups of cruciferin subunits (cru1, cru2/3 and cru4) encoded by distinct gene families are present in B. napus (Breen & Crouch, 1992; Rodin & Rask, 1990) and, in Arabidopsis, cru1 corresponds to CRC; cru2/3 to CRA1 (CRA); cru4 to CRB (Rodin et al., 1992).

In contrast, napins belong to the albumin storage protein family and in the seeds of modern rape varieties, they are present in lower quantities than cruciferins (Berot et al., 2005). Mature napins are highly basic proteins (pI ~ 11) and exhibit molecular weights between 12,500 and 14,500. They comprise two polypeptide chains (4,500 and 10,000 Da) held together by two disulfide bonds and are encoded by a multigenic family initially synthesised as a precursor which is proteolytically cleaved to generate mature napin chains.
Methodology
SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was used to generate profiles of soluble seed proteins, in particular the storage proteins cruciferins (12S globulins) and napin (2S albumin). Several methods were attempted and developed for grinding seed, extracting and assaying soluble proteins and for protein separation by SDS-PAGE. A variety of different extraction buffers (Poms et al., 2004) and gel types (reduced/non-reduced Laemmli (Tris-glycine), Tricine, Bis-Tris and NuPAGE ready-made 4-12% gradient gels) were also assessed to achieve improved separation of the subunits for both storage proteins. Total soluble protein was quantified using the Bradford dye assay.

Purified cruciferin from *B. napus*, monoclonal and polyclonal antibodies were obtained from D. Lydiate, Saskatoon Research Centre, Canada. Immunodetection by Western blotting clearly showed variation between lines for the cruciferin alpha subunits.

Results
Initially, SDS profiles were compared with those shown in previously published work (Berot et al., 2005; Hoglund et al., 1992, Murphy et al., 1989; Rodin & Rask, 1992) to aid identification of the cruciferin and napin proteins. However, it was not possible to resolve or quantify bands using Phoretix™ software (Nonlinear Dynamics) due to the presence of multiple co-migrating bands. It was imperative for our analysis that the cruciferin and napin subunits should be identified prior to any further work relating to precise separation and quantification. Variation in cruciferin and napin subunits can be observed by SDS-PAGE and immunodetection by Western blotting (Figures 6 and 7).

![Figure 6. SDS-PAGE (15% Tris-glycine) and Western blot demonstrating variation in cruciferin and napin content in seed of selected BnaDFS accessions. Cruciferin (Cα=alpha; Cβ=beta) and napin (N(H)=heavy, N(L)=light) subunits are indicated.](image)

![Figure 7. Western blot showing variation in cruciferin subunits of A: representatives of BnaDFS (1-9) and in other *Brassica* species (10-13) and B: between TapidorDH and Ningyou7, parents of the TNDH population. The polymorphism observed between the parental lines of the TNDH mapping populations (Figure 7B) segregated within the progeny enabling us to establish a map location to *B. napus* chromosome A1. We also assessed the feasibility of using ELISA assays for quantification of specific seed proteins, and determined that this would require further optimisation.](image)
Conclusions
We have successfully demonstrated that it is possible to carry out screens for variation in the major Brassica seed proteins and modified existing techniques to characterise genetic loci associated with specific sub-types, making use of monoclonal antibodies previously developed.

Further definitive identification, and characterisation of putative protein bands for both cruciferin and napin subunits will require isolation from SDS-PAGE gels that have been subjected to in-gel trypsin digestion, followed by LC MS/MS or MALDI-TOF/Q-TOF mass spectroscopy analyses (Devouge et al., 2007).

We conclude that the use of SDS-PAGE to quantify the cruciferin/napin ratios amongst different germplasm is not straightforward. However, preliminary data obtained for cruciferin:napin ratios ranged from 0.35-1.56, subject to definitive identification and is comparable to the 0.6-2.0 reported previously (EU-ENHANCE final report, QLK5-1999-01442, 2003). For more accurate quantification, our results indicated that two extraction procedures may be required, with additional purification steps to remove co-migrating bands, for cruciferin and napin, respectively. Furthermore, since extracted samples deteriorate upon storage, it is necessary to subdivide (aliquot) extracts to avoid subjecting them to freeze/thaw cycles.

An alternative approach was performed with 64 OSR genotypes (Malabat et al., 2003), where wide variation was observed for cruciferin (32-53%) and napin (25-45%) content. The procedure involved the use of gel filtration via liquid chromatography to fractionate crude extracts obtained from a three-step purification procedure, followed by quantification of peak areas corresponding to cruciferin, napin and lipid transfer proteins.

WORK PACKAGE 2. Ensure that integrated datasets and information relating to specific crop traits, associated genetic variation and reference resources in OSR are available in a form that is readily usable by the research and stakeholder communities, and that can enhance decision making in relation to trait prioritisation.

2.1 DATABASE CURATION
In order to maximise the impact and take-up of existing and new information and resources from the OREGIN network by the R&D community and plant breeders, it is important that a well integrated data management system is provided that links the various components of OREGIN and associated projects. It is important for long-term value to be drawn from accumulated datasets and that the effort within OREGIN focuses on structured data curation. This is being facilitated by optimising and making use of existing databases developed and maintained at RRES.

Methodology
The appointment of a data curator at RRES dedicated to collation of genetic and associated data relating to Brassica species has enabled a thorough revision of existing database structures and development of a curation pipeline. The work has involved a comprehensive review of the CropStore database tables, with considerable effort to ensure consistency in description of data fields across the database, as well as the ability to ensure clear attribution of data provenance.

A series of Excel workbooks have been edited to provide input templates that are now distributed to laboratories generating data. This provides a ready means of collating and checking data prior to entry into a MySQL database. The underlying database server is maintained and archived at RRES, and work is underway to provide simple interactive querying via the www.brassica.info website for delivery of datasets via web services.

Results
As part of an ongoing international effort led by RRES in agreement with the Multinational Brassica Genome Project work has been carried out to develop and implement standardised nomenclature conventions for plant populations, genetic marker loci and linkage groups. This will provide considerable added-value to OREGIN resources in the context of information provided from the Brassica diploid genomes and sequencing initiatives.

The Input Template Workbooks have been tested with different datasets, and used to populate the CropStore database.

Standard Operating Procedures have been developed to describe the various stages of the curation process and will be posted on the OREGIN and www.brassica.info websites.
Conclusion
The work has focused on refining and harmonising the database structures, and standardised methods of data acquisition and curation. This will improve the ability of researchers and breeders to collectively assess the ability to identify relevant genetic variation and determine the likely downstream work involved in detailed genetic characterisation and resolution prior to exploitation in commercial breeding programmes.

- Consensus input template Excel workbooks are available for download from the websites
- Datasets are validated to source (including provenance)
- Where appropriate internationally agreed nomenclature conventions have been adopted (e.g. linkage groups, marker loci, population names). In many cases, OREGIN is leading the international effort for such standardisation, and contributes to the international repository/registry relating to *Brassica* genetics.

2.2. DATA DELIVERY

Methodology
Data sources were identified and data checked against available published information. Care was taken to accumulate as much relevant descriptive ‘metadata’ associated with different datasets as possible.

It was decided that the most efficient means for delivery of OREGIN plant-based data was to provide a series of links from the ‘resources’ page of the OREGIN website to the relevant pages and database interface managed under the www.brassica.info website. This means that OREGIN users have access to data in a consistent format for OSR as well as diploid brassicas. We have developed a staged approach to delivering the relevant datasets. The first is to provide summaries of available data in static tables on the www.brassica.info website, and more detailed Excel workbooks with detailed datasets (generated from queries of the CropStore database). More recently we have been working with bioinformaticians at RRES to generate a more sophisticated interface. This enables the end-user to select from a small number of sub-categories (e.g. population name, parent line name, population type), following which a summary table is displayed interactively from a web service run on the CropStore database. The user is then able to select one or more datasets for delivery via dynamically generated Excel workbooks. This provides a very flexible means of data delivery.
Results
The datasets are versioned so that there is a clear distinction between different subpopulations that have been described for different projects or end use. The data include description of the BnaTNDH mapping population (including > 4 sub-populations) and associated linkage maps; the populations, the TVSL substitution lines, the *B. napus* Diversity Foundation Set (BnaDFS), including detailed line information. In addition, detailed drill-down data associated with sets of genetic markers (e.g. Microsatellite SSRs and RFLPs) are allowing comparative genomic analysis (OSR to Arabidopsis) and large-scale genetic map integration.

Conclusions
Data are now curated to a high quality, providing long-term added value for geneticists/breeders.
- Data summaries and detailed datasets are delivered via a simple web interface and will allow direct comparison of accumulated datasets in future.
- Datasets associated with the resources developed within the OREGIN project have been checked and collated in a consistent format. A method has been developed for making these data readily available in the public domain.

2.3 Website Maintenance and Development
The OREGIN website (www.oregin.info) was updated regularly to incorporate some results from the OREGIN work and to include presentations and minutes from meetings, such as the Stakeholder Forum (held on 7 March 2007). In addition, the pathogen isolating ordering system was refined and validated to provide access to 110 UK *Leptosphaeria maculans* and 129 UK *Pyrenopeziza brassicae* isolates using a system that generates isolate-specific MTAs on the fly (Figure 9). Isolates from other countries can be requested and can be provided following consultation with the original owner of that isolate.

The www.brassica.info website underwent a complete overhaul during this project period to facilitate navigation of the various resources and information sources, and provide prominent links to the OREGIN website.

2.4 Interactions with OREGIN Stakeholders
The membership of the OREGIN Management Committee was extended to include representation from HGCA levy board, thus providing more direct links to the OSR growing industry, near-end use R&D and trialling activities. In addition, the Management Committee provided the opportunity for more detailed updates, feedback and discussion with the various LINK projects associated with OREGIN and OSR.

In addition, there were updates on the OREGIN network at the annual UK Brassica Research Community meeting, held at Rothamsted in May 2007, as well at other venues.

Figure 9. Material Transfer Agreement (MTA) for three *Leptosphaeria maculans* isolates ordered from the OREGIN culture collection. MTAs specific for the isolates ordered are generated “on the fly” by the database software during the ordering process.
WORK PACKAGE 3. To provide the core OREGIN experimental resources (DFFS sets representing plant genetic diversity, reference mapping populations for genetic resolution, and pathogen collections) available to the OSR R&D and breeding community, together with associated information to enable rapid genetic analysis of sustainability traits.

3.1 CONTINUE SINGLE SEED DESCENT FIXATION OF NON-FIXED BnaDFS FOUNDER LINES
At the start of the project (April 2007) a decision was made in conjunction with the Management Committee to cease attempts at microspore-mediated DH line fixation for recalcitrant lines, and to progress the fixing through a single seed descent (SSD) programme.

Methodology
The agreed plan was to proceed through six rounds of SSD, at which point the lines would be considered fixed (S6 is on average 98% homozygous compared with the starting material). This work is ongoing and is anticipated to progress into the follow-on project. The emphasis at present is to accelerate the process by reducing generation times, partly though seedling stage vernalisation.

Results and Analysis
The rate of progress in getting plants to flower and set seed is slower than originally anticipated, probably resulting from genetic diversity associated with this germplasm. The current status of SSD is that one line has yielded S4 seed, 5 lines have produced S3 seed, 103 lines have produced S2 seed and the remaining 16 lines have given S1 seed. A number of other plants are currently setting seed in the glasshouse. In the next phase of OREGIN the UK OSR breeders have kindly agreed to take on responsibility for completing the SSD programme on most of these lines, for which they have recently received the first batch of seeds.

3.2 BULKING UP SEED FOR GENETICALLY FIXED BnaDFS LINES
The BnaDFS was originally established with 36 fixed founder accessions. Ninety four of the remaining lines were subjected to microspore culture, and of those that responded to produce embryos we have successfully obtained seed from 27 doubled haploid (DH) lines to bring the total number of fixed lines to 63. The majority of these lines have been taken through a round of regeneration over the last year. Elsoms Seeds generously contributed to this task by bulking up 18 lines and 19 lines were regenerated at WHRI. In total we are now in a position to distribute seed for 60 of the 63 fixed lines, and are currently regenerating the remaining 3 lines.

3.3 GENETIC MARKER BASED CHARACTERISATION OF NEW GENETICALLY FIXED BnaDFS LINES
The founder lines of the BnaDFS had previously been screened with a panel of 15 SSRs. In addition, 54 SSRs had been screened against 55 fixed lines. This contributed to the strategy that involves screening batches of fixed lines once they become available. These data provided a baseline measure of genetic diversity and revealed an interesting pattern of crop type-specific allele distribution.

Results and Analysis
The change in strategy to fix lines by single seed descent rather than by the production of DH lines has meant that no new fixed lines became available this year (see 3.1). However, we have extended the marker diversity analysis for nine of the existing fixed lines by screening them with an additional 93 SSRs. These lines were targeted because they are parents of the mapping populations available to OREGIN. The work forms part of a wider study to align and integrate the genetic maps of these OSR populations together with other A and C genome Brassica genetic maps. These data are in the final stages of analysis and will provide valuable background information. Data will be collated and placed on the OREGIN website.

Conclusions
Since it is the fixed lines that will be widely used by researchers and breeders, it is important to carry out a more comprehensive diversity analysis on these lines. The information obtained will be used for (i) a comprehensive analysis of the population structure of the BnaDFS; (ii) to test the feasibility of association mapping with OSR traits - that is, to assign variation for specific traits to underlying QTL at specific regions of the genome, and (iii) contribute to deduction or confirmation of varietal pedigree information.

Further screening of fixed lines as they become available in the future has been scheduled in the subsequent OREGIN project with the aim of screening all the lines with up to five SSRs per chromosome (5x 19 = 95).

3.4 LARGER SCALE BULKING OF TNDH LINES
The complete TNDH mapping population comprises 202 lines, of which 188 have been adopted and made available by OREGIN. Initially a subset of 100 informative lines, established using an early version of the TNDH genetic map, was regenerated on a larger scale for distribution to researchers. The NOVORB LINK project (LK0964) recently identified an overlapping subset of 94 lines based on suitability for use in UK field trials, with a mutually exclusive set of 48 lines.

Methodology, Results and Conclusion
3.5 PRODUCTION OF SINGLE SPORE (SS) SETS OF MOLECULAR MARKER-DEFINED FUNGAL ISOLATES

Fifty virulent *Leptosphaeria maculans* (phoma stem canker) isolates (10 from the UK, 10 from France, 10 from Canada, 10 from Australia, 5 From Germany and 5 from Poland) were selected from the OREGIN culture collection for characterisation (Table 3). DNA extracted from single spore derived mycelial cultures of these isolates was used for estimating the genotypic diversity of the subset by analysing the minisatellite and AFLP banding patterns. Phenotypic diversity was determined by inoculation of *Brassica* differential lines and cultivars with isolate sporule suspensions and assessment of host reaction types on the cotyledons of these seedlings. Twenty-four races were identified in the isolate subset.

**Table 3:** Summary of genetic variation for minisatellite, AFLP and avirulence loci in *L. maculans* isolate subset.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Sample Size</th>
<th>Gene Diversity</th>
<th>Shannon’s Index</th>
<th>Gst*</th>
<th>Nm*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minisatellites</td>
<td>50</td>
<td>0.65</td>
<td>1.49</td>
<td>0.23</td>
<td>1.61</td>
</tr>
<tr>
<td>AFLP</td>
<td>50</td>
<td>0.38</td>
<td>0.57</td>
<td>0.18</td>
<td>2.34</td>
</tr>
<tr>
<td>Avirulence</td>
<td>46</td>
<td>0.44</td>
<td>0.63</td>
<td>0.19</td>
<td>2.11</td>
</tr>
</tbody>
</table>

*Gst*= diversity among populations; *Nm* = estimate of gene flow from Gst

For *Pyrenopeziza brassicae* (light leaf spot), genotypic diversity was estimated from polymorphic bands obtained from AFLP analysis of a set of single spore derived isolates from the OREGIN collection (Table 4). As differential *Brassica* lines do not currently exist for *P. brassicae*, evaluation of phenotypic diversity was not possible. Nevertheless, results of a preliminary study revealed quantifiable differences among 5 *B. napus* cultivars in the levels of host response to isolates of the pathogen.

**Table 4:** Summary of genetic variation for AFLP loci in *P. brassicae* isolate subset.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Sample Size</th>
<th>Gene Diversity</th>
<th>Shannon’s Index</th>
<th>Gst</th>
<th>Nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFLP</td>
<td>155</td>
<td>0.46</td>
<td>0.66</td>
<td>0.05</td>
<td>9.20</td>
</tr>
</tbody>
</table>

3.6 ASSESSMENT OF *B. NAPUS* DIVERSITY AND MAPPING POPULATIONS FOR RESISTANCE TO THE MAJOR FUNGAL PATHOGENS *LEPTOSPHAERIA MACULANS* AND *PYRENOPEZIZA BRASSICAE*.

The field experiment in the 2006/07 growing season consisted of 46 lines/cultivars (historical and current cultivars and material from the BnaDFS) grown in a randomised complete block design with three replicated blocks. Phoma stem canker was assessed on five occasions. The first external symptoms were noted in early March 2007 on Nugget, Drakkar, Liho, Marita, Global, Stellar DH and wild accession. At harvest (early July 2007), internal symptoms were assessed on a 0-4 scale where 0= no symptoms, 1 = 0-25% of stem girdled, 2 = 26-50% of stem girdled, 3 = 51-75% of stem girdled and 4 = >76% of stem girdled or plant dead. The lowest mean severity was recorded for cultivar cv. Hearty (1.8). There were six cultivars/lines with a mean severity score of 4 (Bronowski, Drakkar, Fido, Nugget, Stellar DH and Tapi DorDH). Of the remainder, approximately half had mean severity scores from 2-3 and the other half from 3-4. Light leaf spot was assessed on leaves three times between early February 2007 and late April 2007. Symptoms were slow to develop and patchy (the experiment was not inoculated), but a good assessment was made in April 2007 on a 1-9 scale where 1 is the most susceptible and 9 the most resistant. There were three cultivars/lines that showed no symptoms (*P. brassicae* assexual sporulation) on this occasion: Elan, Global and Wild Accession and the most severe symptoms were observed on cvs Canary and Darmor (mean score of 4.3). Light leaf spot symptoms on the stem were assessed at harvest. No symptoms were observed on 19 of the 46 cultivars/lines. On the remaining cultivars/lines, mean symptom severity ranged from 0.17% stem cover (cv. Canary) to 3.38% (cv. Bristol).

As in previous growing seasons, historical and current cultivars and BnaDFS material (42 cultivars/lines) were grown in an experiment to monitor resistance response to the major winter oilseed rape pathogens *Leptosphaeria maculans* (phoma stem canker) and *Pyrenopeziza brassicae* (light leaf spot) during the 2007/08 growing season. The experiment was sown in August 2007 and assessed at regular intervals for the development of phoma leaf spot and stem canker (*L. maculans*) and light leaf spot (*P. brassicae*). Material gave a wide range...
of responses from very susceptible to highly resistant but different lines gave consistent responses to those observed in previous seasons.

Assessments of phoma leaf spot began in October 2007, when symptoms were visible on 15 out of the 42 cultivars/lines (highest mean severity of 3.33% leaf area on cv. Jet Neuf). Symptoms peaked in early February 2008, when mean symptom severity was lowest on Janetzki Schlesischer (2.33% leaf area) and Hearty (2.67% leaf area) and highest on Nugget (13.33%), Drakkar (10.67%) and Apex (10.33%). Mean disease incidence was highest on Akela (93% of plants symptomatic) and lowest on Emerald and Lioness (20% of plants symptomatic). Light leaf spot symptoms were first observed on leaves in mid-January on cvs Bristol and Quinta. The increasing to 100% by mid-March. In mid-March 2008, the highest mean symptom severity was observed on Hearty (33.3% leaf area), Jet Neuf (30% leaf area) and Bristol (30% leaf area) and the lowest on Sarepta (5.3%), Elan (5.7%) and Mohican (6.3%). Of the 42 cultivars/lines sown this season, 23 were also sown last season. There appears to be some correlation between the two seasons for phoma symptoms on individual cultivars, but not for light leaf spot symptoms.

Standard Operating Procedures (SOPs) for field experiment design and disease assessment observations were uploaded to the OREGIN website.

WORK PACKAGE 4. To provide a basis for environmentally sustainable agriculture in relation to predicted climate change by characterising the OREGIN biological resources in the context of adaptations to climate change and modelling predicted effects of climate change to inform future policy, breeding and farming decisions.

4.1 Characterise effects of predicted climate change on host-pathogen interactions of OSR fungal diseases.

A weather-based model developed to predict the start and severity of epidemics of phoma stem canker (Leptosphaeria maculans) was combined with data produced by a program to generate runs of likely weather data under different climate change scenarios to model the likely effects of climate change on phoma stem canker epidemics. The model predicted that warmer winters would significantly advance the date of stem canker appearance in spring and increase the severity of canker before harvest. The model also predicted that epidemics will spread north from England to Scotland, where cankers do not currently occur on oilseed rape (Figure 10).

Figure 10. Predicted severity of phoma stem canker (L. maculans) at harvest on winter oilseed rape crops (mean of resistant and susceptible cultivars) for (a) baseline 1960–1990, (b) 2020LO, (c) 2020HI, (d) 2050LO and (e) 2050HI climates. Stem canker severity was assessed on a 0–4 scale (0, no disease; 4, plant dead; Zhou et al. 1999). Areas unaffected by the disease are white.

A paper (Range and severity of a plant disease increased by global warming, Evans, N., Baierl, A., Semenov, M.A., Gladders, P., Fitt, B.D.L., (2008). JRSI, 5, 525-531) was published in the Journal of the Royal Society Interface online in August 2007 and in print in June 2008. A similar approach was taken for light leaf spot (Pyrenopeziza brassicae) which indicated that light leaf spot epidemics would become less severe with global warming to the extent that in the south of the UK, the disease would disappear.
**FUTURE WORK**
The principal activities of the OREGIN project are the generation, gathering, collation and dissemination of information and genetic resources for the benefit of the stakeholders. The OREGIN network has been successful in achieving initial objectives of: a) providing a focus for the UK OSR genetic improvement R&D and stakeholder communities and the means to prioritise research requirements; b) initiating the identification of genetic diversity within the crop gene-pool and the gene-pools of major UK pathogens and establishing structured archived resources for their characterisation and exploitation. This has required considerable consultation and involvement between the original contractors and others involved in delivering R&D. LINK and other funding schemes have provided a conduit to the private sector breeders who ultimately are best-placed to deliver new cultivars into the market place. The recent inclusion of a representative from HGCA (AHDB) into the Management Committee allows greater interaction and more direct dialogue between those involved in crop improvement and agricultural practice.

It has been agreed that future work should concentrate on delivering a breeders and pre-breeding 'toolkit'. This requires i) genetic markers in the context of reference linkage maps; ii) a defined range of germplasm representing significant, useful allelic variation; iii) information to place these resources in context of trait variation amenable to breeding selection. It is anticipated that this will be achieved via the following objectives:

1. Maintain and develop OREGIN seed and pathogen genetic resources, and enable resolution and introgression of useful variation
2. Quantify and characterise genetic variation for key traits
3. Delivering the pre-breeding toolkit: provision of integrated information to maximise utilisation of genetic diversity

Particular emphasis will need to be placed on data collation and integration, as this underpins all relevant activities by researchers, breeders and associated LINK consortia.

**ACTION RESULTING FROM THE RESEARCH (IP, KNOWLEDGE TRANSFER)**

The primary delivery from the OREGIN project is Knowledge Transfer, free of IP. The resources and data are made available via the public domain, and future added value will arise from integration with datasets generated in LINK and other projects in UK and elsewhere.

**References to published material**

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.
Papers in Refereed Journals


Trade press


Proceedings of Conferences, meetings


**Oral presentations**

Barker G and Teakle GR (25Jun2007, broadcast week of 01Oct07), iCAST on Biofuels from brassicas: [http://www2.warwick.ac.uk/newsandevents/icast/archive/s2week1/brassicas/](http://www2.warwick.ac.uk/newsandevents/icast/archive/s2week1/brassicas/)

King GJ Integration of genetic and genomic knowledge for Brassica oilseed productivity. NRC-Plant Biotechnology Institute, Saskatoon Canada, July 26th 2007.


King GJ Integration of genetic and genomic knowledge for Brassica oilseed productivity. IVF, Chinese Academy of Agricultural Sciences, Beijing, China, Oct 2007.


Pink DAC gave a presentation at the Midlands Biomass event at Moreton Hall 15 November 2007 - The Future – Technical potential “Issues and opportunities”

Teakle GR, Warwick HRI department seminar, Nov 2007 – Genetic diversity in Brassicas

Teakle GR. Short presentation entitled “Nitrogen use efficiency in oilseed rape” at the UKBRC meeting, Warwick HRI, 21 May 2008

Teakle GR. Short presentation entitled “Genetic resources and map integration” at the UKBRC meeting, Warwick HRI, 21 May 2008

**Websites**

http://www.oregin.info
http://www.brassica.info/resources.php
## Appendix 1

**Updated Summary of ongoing funded projects related to OREGIN (2007-8)**

### SUMMARY OF NEW FUNDED PROJECTS RELATED TO OREGIN

**June 2008**

Total funded projects:

<table>
<thead>
<tr>
<th>Project Title (dates)</th>
<th>Funding Body</th>
<th>Partners</th>
<th>Brief Summary</th>
<th>Budget/ contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB/F009712/1 Modulating Seed Size in Oilseed Rape (2008-13)</td>
<td>BBSRC</td>
<td>Rothamsted Research, University of Bath</td>
<td>Determine genetic and mechanistic basis for changing seed size, which plays a key role in the yield, quality and profitability of OSR, with ancillary effects on crop establishment</td>
<td>£528,707</td>
</tr>
<tr>
<td>BBF0157981 Reducing the carbon footprint of the lubricants industry by the substitution of mineral oil with rapeseed oil (2008-13)</td>
<td>BBSRC/LINK Renewable Materials</td>
<td>JIC, Warwick HRI</td>
<td>To underpin the development of oilseed rape varieties for the production of oil for use in the lubricants industry</td>
<td>£433,762</td>
</tr>
<tr>
<td>IF0128 The impact of shortened rotations on rhizosphere microbial diversity (2007-2009)</td>
<td>Defra</td>
<td>Warwick HRI</td>
<td>Establish the impact of growing OSR in shorter rotations on the soil microbial community in relation to yield</td>
<td>£283k</td>
</tr>
</tbody>
</table>