

## **Genetics of Seed Quality Traits (Objective 6, March 2007)**

*This will involve*

- a) expansion of Objective 1 to include industrial end users within the range of interested parties*
- b) wide consultation with a wide range of industries to define priority seed quality traits*
- c) development of tools and definition of protocols for analysis of these traits*
- d) establishment and analysis of recombinant inbred populations that segregate for key traits in relation to seed and protein quality, as defined by these end-users*

### **PROGRESS:**

This objective links closely with Objective 1 and depends on interaction with the wider stakeholder group that includes end-users. These links have been achieved and are the product of ongoing activities (see Objective 1). The definition and prioritisation of target traits were based on wide consultation, with the overall aim of providing tools and resources to industry. Input was sought additionally from the industry in the choice of lines and protocols for Objective 6. As outlined in the previous report, traits relevant to three end-use areas were defined, with the aim of dissecting the genetics of traits of interest. Based on an understanding of the genetics of these traits, simplified genetic screens for 'field' use will be devised, that are particularly valuable for the screening of seed characteristics at a very early plant stage.

### **A: Animal feed**

Improvements to home-grown sources of animal feedstuffs are relevant to Defra's objectives for improved quality, linked to a reduction in processing, energy use and minimisation of waste. Improved quality in feed, coupled with improved digestibility, will be linked to a reduction of potentially eutrophivating waste, with associated effects on natural resources.

Dr Julian Wiseman, School of Biosciences, University of Nottingham, a specialist in animal nutrition, was involved in discussions on targets for animal feed improvement, and offered to present relevant data at the stakeholders' meeting in September 2006. The scientific information provided by Dr Wiseman, together with the guidance issued by Premier Nutrition, demonstrates that peas can be included at higher rates in pig and poultry diets than is acceptable for either field (faba) bean or lupin. Inactivation of anti-nutritive compounds allows peas to be included at higher amounts (40%) and such diets have benefits for meat quality with reduced taint.

An earlier study of the biochemistry and genetics of a family of anti-nutritional proteins in pea seeds led to a facile DNA marker screen currently in use by two companies. Mutants for these proteins have now been sought, using the platform available through the EU GLIP project. The sequence of the TI1 genes encoding the pea seed trypsin-chymotrypsin inhibitor proteins has been exploited to screen and

identify mutants in genes with the aim of generating pea lines with very reduced amounts of anti-nutritional proteins.

Three mis-sense mutations giving rise to amino acid changes have been identified:

**C77Y:** a highly conserved cysteine residue has been replaced by a tyrosine.

**S85F:** the active site serine in the chymotrypsin inhibitory loop has been replaced by a phenylalanine.

**E109K:** a glutamic acid residue in the carboxy-terminal extension has been replaced by a lysine, an alteration in overall charge in this region of the protein that is subject to post-translational processing.

Heterozygotes were identified for the first two mutant classes but the single heterozygote for the second mutant showed an abnormal phenotype, with very bushy growth over a prolonged period and no flowering. Eventually, pods were formed with very thick walls but no seeds developed. Further seeds were requested for this line and heterozygotes identified. The progeny seeds have been sown for further screening. For the last mutant, 3 homozygous lines were identified. Bulked seeds from mutant lines will be used for protein analyses. The mutant lines will be back-crossed to the parent, cultivar Cameor.

## **B: Food crops**

An understanding of the scientific definition of food quality is linked to Defra's overall objectives for improved food quality and nutritional value, coupled with a reduction in processing and waste. More generally, improved quality selection procedures will lead to more efficient supply chains, with reduced energy use, waste and carbon emissions. The problems outlined below can lead to the use of undesirable chemicals. Biological solutions to these problems therefore contribute directly to Defra objectives for improved food quality and nutritional content, together with waste minimisation.

The development of tests to facilitate breeding for the food industry, based on the identification of metabolites involved in quality, was discussed in last year's report and at meetings. NMR analysis and metabolite profiling of mature seeds of a vining pea cultivar (*cv.* Puget) are being pursued within the satellite project at CSL, York (represented by Adrian Charlton at PCGIN meetings). Robust protocols for seeds have led to the identification of metabolites that respond to the environment as well as to genotype. These data are being analysed currently.

In last year's report, the various problems that have affected pulses in recent years were discussed. Some aspects of research on bruchid pests in pea were discussed, in relation to the growing problem in UK with this pest in beans. These discussions, along with those at the PCGIN stakeholders' meeting in September 2006 that included an invited speaker from France to provide an update on research, led to a number of PCGIN initiatives on *Bruchus rufimanus*, as outlined under Overview (above).

In pea, some natural resistance to *Bruchus* (*Bruchus pisorum*) has been reported to be attributable to the locus, *Np*, where neoplastic growths form on pods in response to (among other stimuli) chemicals from the bruchid eggs. Lines of pea carrying *Np*

were included in a display of genetic material at the stakeholders' meeting in September, 2006. Seeds from two such lines have been passed to Nickerson-Advanta, and there was general interest from the industry in becoming involved in research in beans to identify similar resistance mechanisms (Velcourt Ltd, Lincolnshire).

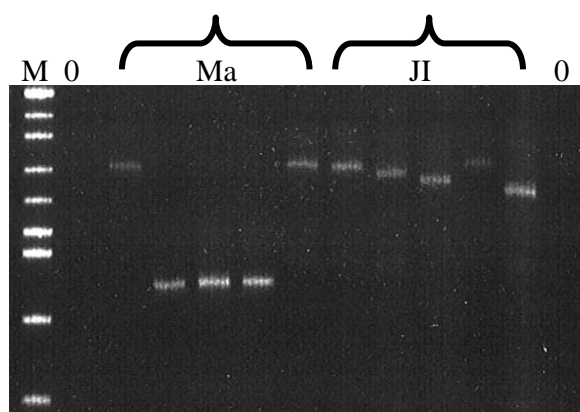
A second source of natural resistance has been reported in *Pisum fulvum* accessions. To investigate this, an M.Sc. student project was proposed that aimed to explore natural variation in defensin genes in pea, with emphasis on diversity in *P. fulvum* accessions. The Australian resistant line is being included in this ongoing study.

Genetic loci involved in marrowfats seed quality were identified as targets, as discussed in last year's report, and those implicated in colour retention have been pursued, as described in sub-sections 1-3 below.

1. Impact of variation in seed lipoxygenase activity on seed colour retention:

A pea line, L-3, having reduced amounts of lipoxygenase-3 (LOX-3), has been crossed with the range of marrowfat lines supplied by PGRO as representative of those being used by the industry, and showing the undesirable seed bleaching problem (5 lines; see below). L-3 is a yellow-seeded line, so to assess the possible effect of the *Lox* gene variant on green pigment stability, all F<sub>2</sub> seeds were screened first for cotyledon colour. A proportion of seeds that had the recessive *i* allele (green) were sown and F<sub>2</sub> plants screened for *af* (semi-leafless gene in four of the marrowfat parents) and *Lox* gene variants. Due to linkage between *i* and *af*, an excess of semi-leafless plants was recovered from the seeds sown.

Primers based on the *Lox-3* gene promoter were designed in order to develop a marker for the gene(s). An example of the *Lox* gene variation is shown for a number of pea lines in Figure 6.1, with considerable size variation in the amplified promoter fragment (approx. 350 to 700 bp in length). Among F<sub>2</sub> plants, both parent and heterozygous *Lox* alleles were identified, due to the co-dominant nature of the gene marker. The numbers of F<sub>2</sub> plants scored as homozygous for the L-3 *Lox* allele are given below for four crosses (as a fraction of the numbers screened).



**Figure 6.1: Variation in *Lox-3* gene fragments amplified from 10 pea lines using a pair of promoter primers: 5 marrowfat lines, Ma; 5 genotypes from the JI germplasm collection, JI (0 = no DNA control; M = marker)**

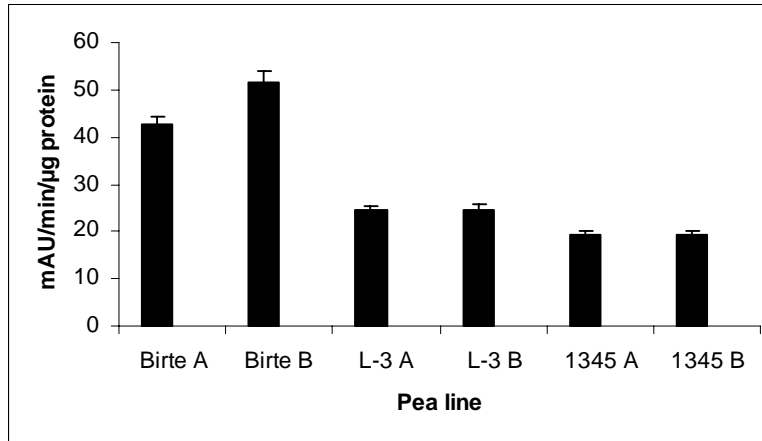
**F<sub>2</sub> seed/plant scores**

<b>L-3 x</b>	<b><i>i:I</i></b>	<b>L-3 <i>Lox</i> allele</b>
Princess	110:287	3/9
Kahuna	24:48	5/9
Orka	39:95	3/9
Samson	5:29	1/3
Maro	3:14	

Maro is a leafed cultivar that, although highly favoured for seed quality, has several agronomic problems and has now been removed from the recommended list (NIAB/PGRO); therefore, this cross has not been pursued further.

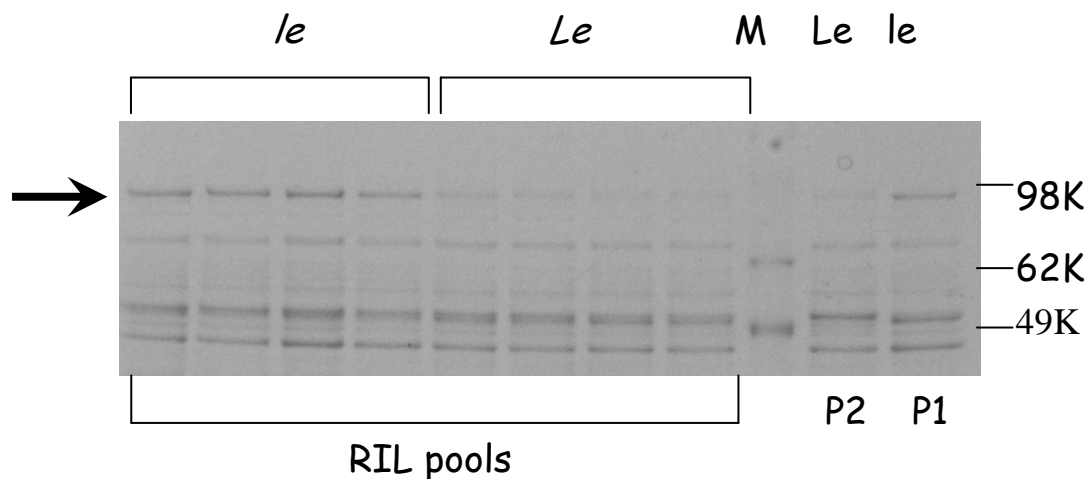
For the first three crosses above, F<sub>3</sub> seeds from F<sub>2</sub> plants of the genotype *i, af*, L-3 LOX will be sown for back-crossing to their respective parent. BC1 F<sub>2</sub> seeds will be assessed for pigment bleaching in relation to the LOX allele. For the cross involving Samson, the homozygote for the L-3 *Lox* allele was an *Af* segregant; if this is a heterozygote, an *af* progeny line will be selected for back-crossing. Otherwise, *af* will be selected from the BC lines in combination with the two segregating variant *Lox* alleles.

A second pea line having reduced LOX activity was identified that showed a reduction in the relative intensities of both *Lox-3* and *Lox-2* proteins following gel electrophoresis of fractionated seed protein extracts. The relative enzyme activity in this line (JI 1345, with a wild, tall phenotype and yellow cotyledon colour) was lower than that of the line, L-3 (Fig. 6.2).



**Figure 6.2: Lox activity in seeds from the pea lines, Birte, L-3 and JI 1345. A, B = biological replicates; SD of replicate assays**

Figure 6.3 shows quantitative segregation of Lox (approx. 95000 molecular weight) in recombinant inbred (RI) segregant classes derived from a cross between the cultivar Birte (P1) and JI 1345 (P2). The combined data indicate that quantitative control of the amount of Lox protein and activity is linked to *le* (internode length), close to the structural genes. Therefore, a marker based on the structural gene(s) would facilitate selection of segregants for this quantitative trait locus (QTL).



**Figure 6.3: Quantitative variation in the amount of Lox protein (arrow) in progeny lines, compared with parents (P1, P2). M = Molecular weight markers; *Le*, *le* = internode length**

Variation in the promoter sequence, as shown in Fig. 6.1, was used to follow the segregation of *Lox* among the RI lines. Recombinants having the *Lox* allele (JI 1345) responsible for reduced activity were sought among the short (*le*) lines. Two lines

(H29 and H53) were selected as having desirable alleles at three loci involved in seed protein quality, including *Lox*. These lines will be crossed to three of the marrowfat cultivars (Princess, Kahuna, Samson) and progeny selected for green-seeded cotyledon colour in order to assess the effect of low *Lox* on colour retention.

2. Impact of a 'supergreen' phenotype on seed colour retention:

A visual screen of seeds from lines representative of the John Innes germplasm collection led to the identification of two pea lines having an intense green colour, which appeared to have been stable in the stored seed samples. Seeds from the two lines were sown for crossing to the marrowfat lines, Princess, Kahuna and Samson, giving rise to 6 crosses. (Kahuna has been retained as a common parent here and for *Lox* crosses, as this cultivar is also one of the parents in the three-way cross in Objective 3, where many markers will be mapped).

Primers based on the candidate gene for *i* (cotyledon colour) have been used to investigate allelic variation at this locus within germplasm accessions and marrowfat lines. Preliminary studies suggest variation for this gene within green-seeded lines (not shown). Inheritance of gene variants in the crosses will reveal whether or not this variation impacts on the activity of the enzyme involved in breakdown of colour pigments.

The nature of the 'super-green' phenotype will be followed in crosses involving JI 813 and other JI parent test lines, facilitating allelism tests.

3. Impact of an orange pod phenotype on seed colour retention:

An orange-podded line, JI 1573, has been crossed to the marrowfat line, Princess, as well as to JI parent test lines. The pigmented pod may provide greater protection of seeds from intense light, and the inheritance of this character will be monitored.