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**SID 4**

# Annual/Interim Project Report for Period 2010/2011

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## Project details

1. Defra Project code: IF0147
2. Project title: Defra Pulse Crop Genetic Improvement Network
3. Defra Project Manager: Dr Farhana Amin
4. Name and address of contractor:
   John Innes Centre
   Norwich Research Park
   Colney
   Norwich
   Postcode NR4 7UH
5. Contractor's Project Manager: Noel Ellis & Claire Domoney
6. Project: start date ..... 01/02/2009
   end date ..... 31/01/2014

## Scientific objectives

7. Please list the scientific objectives as set out in the contract. If necessary these can be expressed in an abbreviated form. Indicate where amendments have been agreed with the Defra Project Manager, giving the date of amendment.

1. Trait biology, to understand the genetic control of key traits relevant to plant performance and seed quality. This will be based on detailed phenotypic analysis under field conditions of pea populations segregating for economically important breeder priority traits. A minimum of three traits will relate to plant agronomy, while seed quality traits will be scored in the crosses involving vining and marrowfat parents. Linking these traits to genetic maps as quantitative trait loci with adjacent marker and primer information will provide tools directly to breeding programmes. The outputs will have application to related pulse species, where markers may be transferred in consultation with stakeholders.

1.1 To understand the genetic control of yield components traits using PCGIN RIL populations
1.2 To generate RIL populations as a resource for the study of key traits in vining pea
1.3 To understand the genetic control of key seed quality traits using PCGIN RIL populations

2. Genetic mapping, to provide reference maps for marker analysis of the traits under investigation in 1, and additionally support the selection of lines carrying desired traits in the wide crosses established for pea; to build on international links and resources to provide for genetic mapping in faba bean for UK benefit.

2.1 To provide genetic maps for PCGIN RIL populations to underpin QTL analyses
2.2 To provide for genetic marker development in pea
2.3 To provide for genetic marker development in faba bean
2.4 To provide updates on genetic marker development in lupin

3. Genetic resources, to expand the available resources for pea and faba bean, exploiting collaborations with European platforms for pea, and international collections of faba bean.

3.1 To provide novel genetic resources for pea
3.1.1 To obtain novel mutants associated with quality traits
3.1.2 To obtain novel mutants associated with performance traits
3.1.3 To generate NILs for QTL identified by areas 1 & 2
3.1.4 To analyse fast neutron deletions affecting plant architecture

3.2 To expand the genetic resources for faba bean in UK
3.2.1 To establish faba bean inbred lines
3.2.2 To provide phenotypic descriptors of faba bean lines and accessions
3.2.3 To establish mapping populations for faba bean

4. Management & communication, to provide for communication channels, based on established PCGIN communication networks with breeder and end-user communities, and exploiting EU and wider industrial connections.

4.1 To manage PCGIN in a responsive manner
4.1.1 To establish related programmes of work

4.2 To integrate PCGIN with international activities

4.3 To disseminate and publish results

## Summary of Progress

8. Please summarise, in layperson's terms, scientific progress since the last report/start of the project and how this relates to the objectives. Please provide information on actual results where possible rather than merely a description of activities.

Scientific progress, with reference to milestones (M):

**1. Trait biology**

**1.1 To understand the genetic control of yield components traits using PCGIN RIL populations**

Mapping genes controlling yield in combining pea populations

The recombinant inbred lines (RILs) derived from the crosses Brutus x Enigma (BE), Brutus x Kahuna (BK) and Enigma x Kahuna (EK) were taken through from $F_8$ to $F_{10}$ seed during 2010 by single seed descent (SSD). Currently the number of RILs at $F_{10}$ is 200 for BE, 205 for BK and 178 for EK. The $F_{10}$ plants will be used for DNA collection and further map development, enabling the bulking of $F_{11}$ RIL seeds for general distribution and trials.

The progeny $F_8$ seeds from the $F_7$ bulks (reflecting $F_6$ RILs) were multiplied in plots at NIAB (BK and BE populations) and at PGRO (EK) to give $F_9$ bulked seeds that will be used for the 2011 plot trials at both locations (see below).

For the EK/KE lines, some of the data acquired for the 2010 PGRO plots are summarized in Figure 1.1.

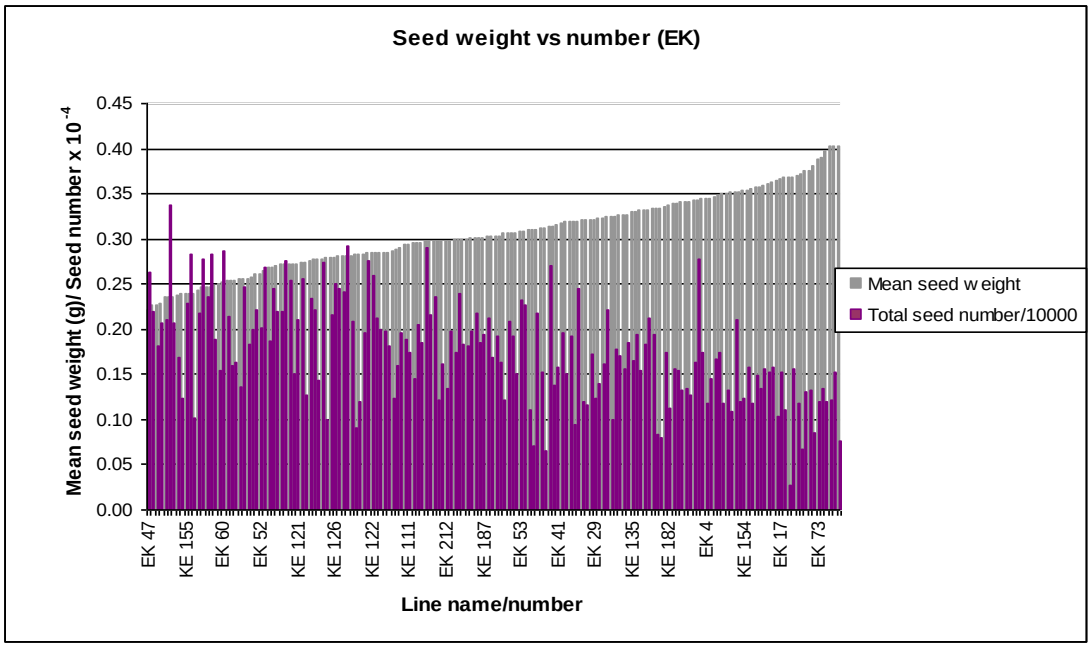



*Figure 1.1: Mean RIL seed weight and total seed number derived for plots of RILs for Enigma x Kahuna*

As expected, there is quantitative variation in mean seed weight among the RILs, in a range that reflects the means for the parents Enigma and Kahuna (0.25g and 0.37g, respectively). An inverse relationship between mean seed weight and overall seed number for RIl plots is indicated in Figure 1.1 but, as these data are derived from single unreplicated plots, they must be considered preliminary and approximate. Nonetheless, any lines showing consistent deviation from such a relationship will be candidates for improved yield, and data linked to the QTL that will be defined, based on the triplicated plot data in 2011. Of the164 EK/KE lines that have been bulked, all apart from14 have in excess of the required number of seeds for triplicate plots in 2011 (see below); for these 14, smaller or fewer plots will be sown.

For the BK/KB lines at NIAB, problems with drilling and combining in 2010 led to a loss of some lines and seeds from other lines, at the two respective stages of the trials. However, variation in seed size among the RILs is again apparent, in a range that reflects the means of the two parents, 0.24g for

Brutus and 0.33g for Kahuna (Figure 1.2).

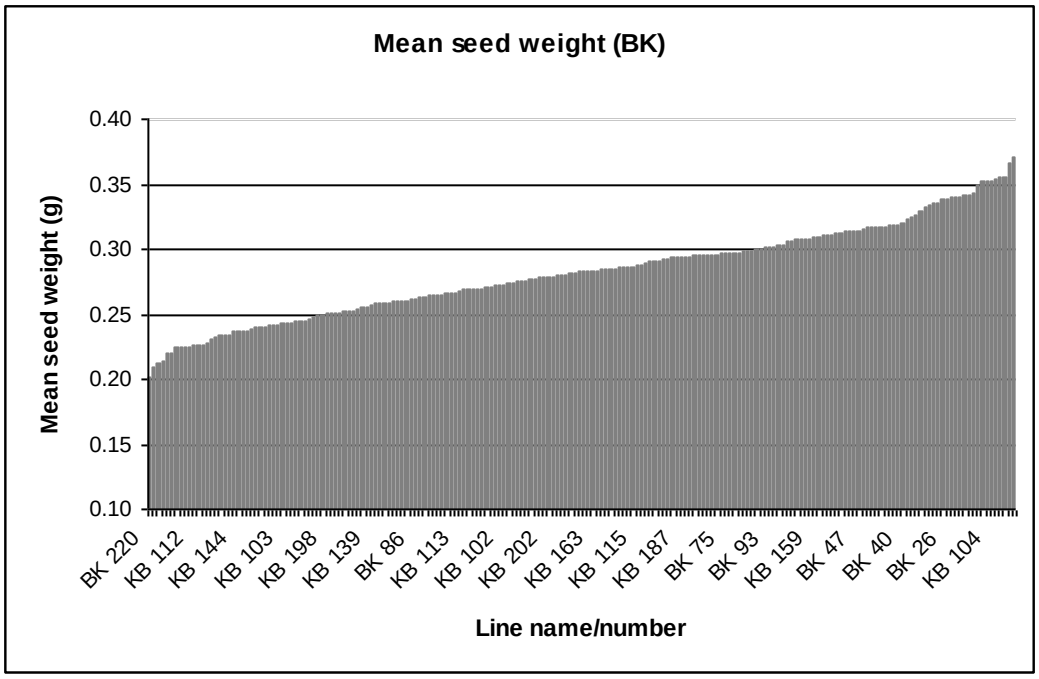



*Figure 1.2: Mean seed weight derived for RILs from Brutus x Kahuna*

The BE/EB RILs were also grown at NIAB. Here, although the parents have seeds of similar size (0.22-0.23g), some variation in seed size among progeny is apparent (Figure1.3), possibly indicative of segregation of the different QTL described for this trait (see below, Table 2.1). The seed yield per plant for this population showed variation among RILs, with one significant outlier (Figure 1.4). Again, these data are obtained from single plots per line and more robust data will be derived from the 2011 plot trials of all three populations (NIAB and PGRO).

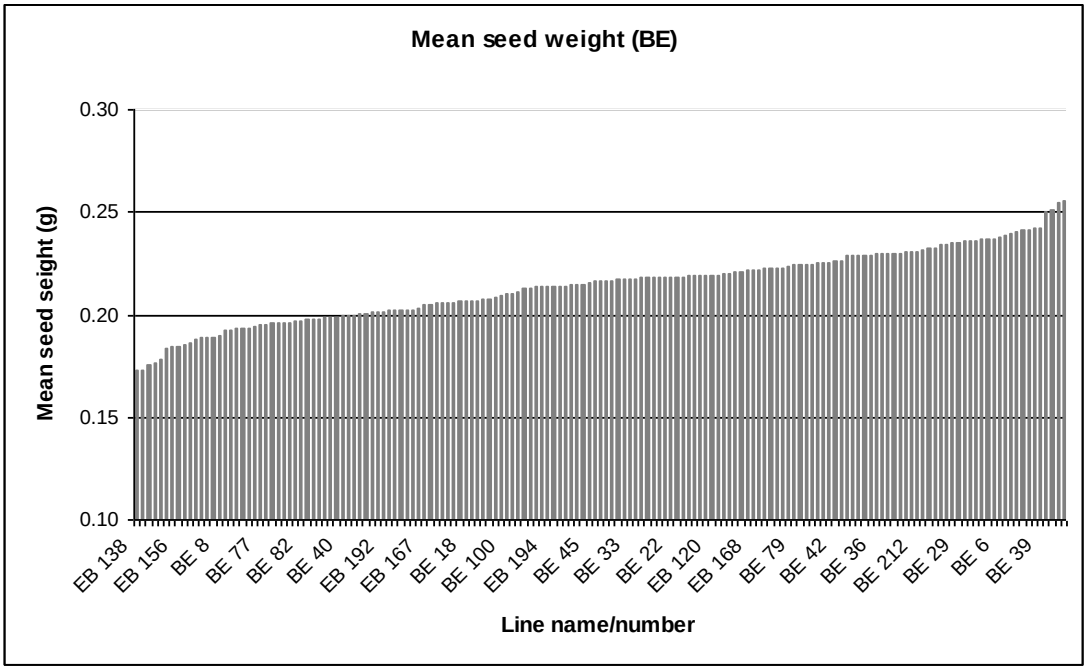



*Figure 1.3: Mean seed weight derived for RILs from Brutus x Enigma*

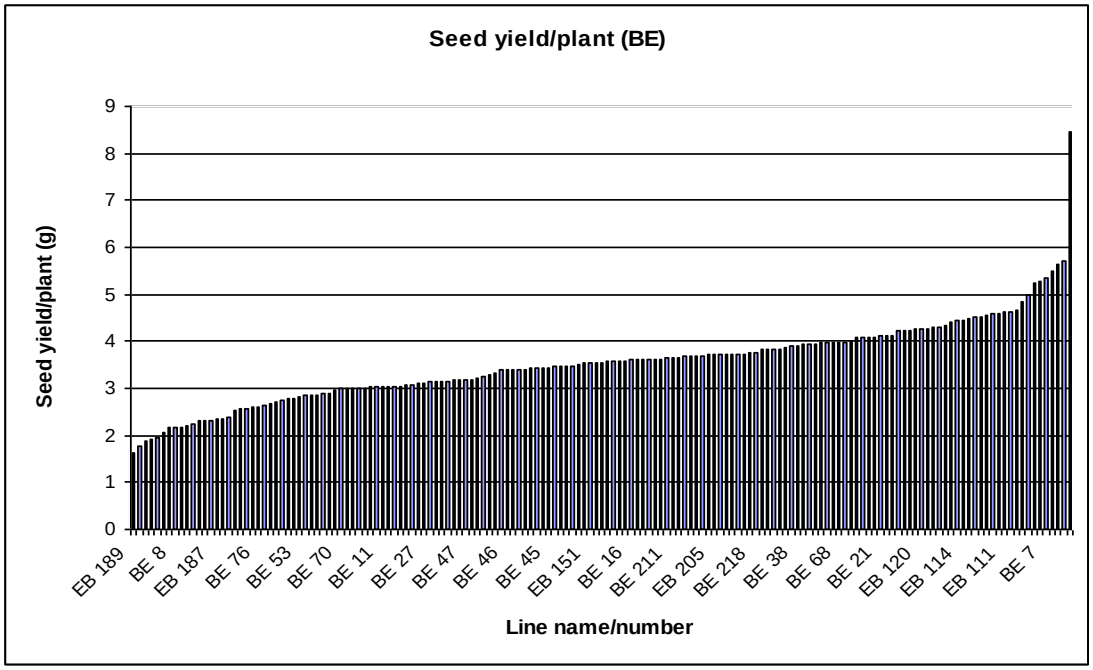



*Figure 1.4: Seed yield data derived for RILs from Brutus x Enigma*

At NIAB, there were clear differences between plots for height, lodging and maturity, and an opportunity was taken to record these, though in the absence of replication the scores should be regarded as preliminary. An example of data from the Brutus x Enigma population for lodging is shown in Figure 1.5, and for maturity in Figure 1.6.

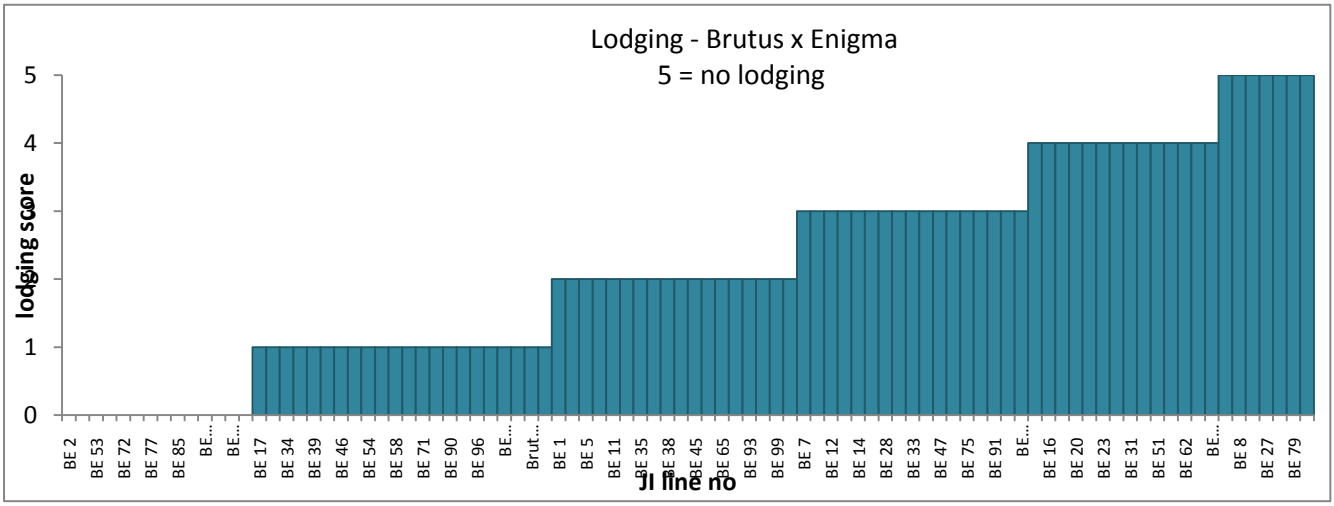



*Figure 1.5 Lodging data for RILs derived from Brutus x Enigma*

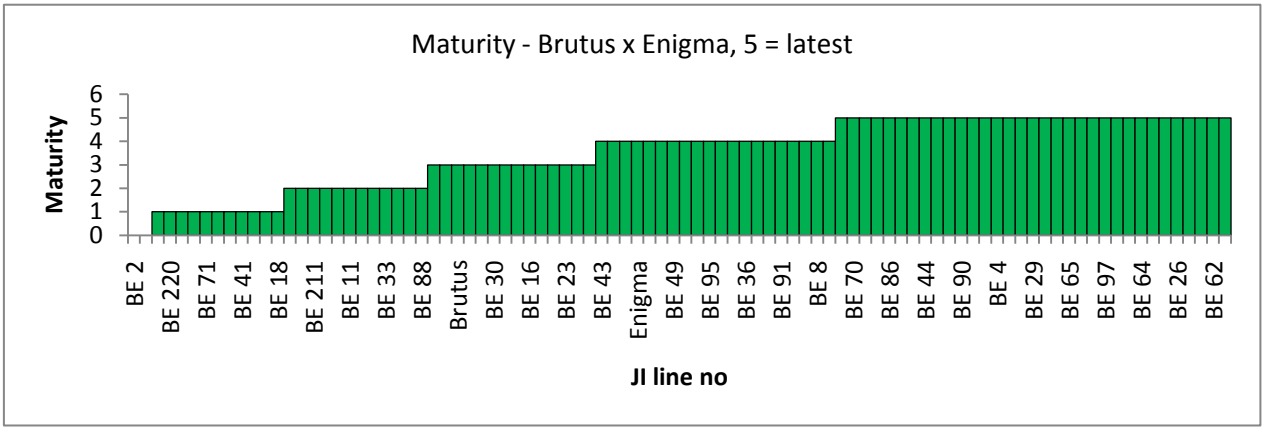



*Figure 1.6 Maturity data for RILs derived from Brutus x Enigma*

There was continuous variation for both characters, parental values being towards the least lodging or earliest maturing scores.

The plots for 2011 will use the F9 bulked seeds from the 2010 F8 plots (and a few lines multiplied at JIC in 2010) [The 2010 plots used the 2009 F7 seeds bulked by PGRO in a polytunnel]. The 2011 trials require 60 seeds per $m^2$, i.e. 360 seeds for every 6 $m^2$ plot (2x3m at PGRO, Thorney site and NIAB, Cambridge site); every RIL will be represented in three replicate plots where possible (1080 seeds per RIL). Of the 196 BK/KB RILs that have been bulked, 26 lines fail to meet the requirement for triplicate plots and these lines will be sown in smaller or fewer plots; the failure is due to the technical problems with equipment outlined above, rather than that the lines are compromised in their performance in the field. All the BE/EB RILs produced seeds in vast excess of the required number. The residual seeds are available for analysis of additional traits (such as 4. below, the subject of a new project proposal application involving the industry).

Traits to be studied and mapped within PCGIN RILs from Enigma, Brutus and Kahuna:
1. Lodging and components
2. Disease
3. Yield
(4. Protein content and additional seed traits from progeny seeds).

The quantitative analysis of these traits will be carried out, based on plot trial data, acquired in 2011 and assembled in excel format in later part of the year.

**1.2 To generate RIL populations as a resource for the study of key traits in vining pea**

The RILs derived from the crosses Avola x Cabree, Avola x Waverex and Cabree x Waverex were taken through from $F_4$ to $F_6$ seed during 2010. However, due to poor seed set and the loss of some plants through premature death, 10% of Avola x Cabree, 33% of Avola x Waverex and 38% of Cabree x Waverex RILs have been re-sown ($F_5$ seed) in a lit glasshouse from November 2010. When seeds from these replacement plants have been harvested, these will be sown and DNA extractions will be done from the $F_6$ plants.

**1.3 To understand the genetic control of key seed quality traits using PCGIN RIL populations**

Single seed descent (SSD) of the RILs has proceeded under glasshouse conditions at JIC. The bulk of the population is now at $F_{11}$, awaiting some catch-up lines which failed earlier. Leaves were harvested from $F_{10}$ plants for DNA preparation, to support genetic map development beyond that generated at $F_6$.

The RI population (150 lines) derived from a marrowfat x supergreen (MxS) cross, that was bulked in Canada during 2009, was further bulked in US (Arizona) winter nurseries in winter 2009/2010 to provide sufficient $F_9$ seeds for field trials in 2010. Samples of seeds from the two field locations were returned to JIC for analysis. Plot trials were conducted in Canada (University of Saskatchewan) in 2010, using triplicate plots for every RIL ($F_{10}$ bulks) at each of two locations. Samples of seeds from two plots at each location are now available for analysis of seed traits, while the remaining seeds will be sown again in 2011 for further replication.

**2. Genetic mapping**

**2.1 To provide genetic maps for PCGIN RIL populations to underpin QTL analyses**

Genetic map construction for seed quality analysis
The genetic map for seed quality characters was enriched with further gene-specific markers, with an emphasis on genes implicated in the chlorophyll degradation pathway. Candidate lipoxygenase *(Lox*) genes have not been mapped in the MxS cross, due to a lack of polymorphisms in coding sequences, introns, or in immediate non-coding regions. It remains possible that there are promoter sequence differences further upstream.

Quantitative data for seed traits were collated from the MxS RILs at $F_8$ (SSD lines, glasshouse), $F_8$ (field, Canadian bulk multiplication plots) and $F_9$ (field, US bulk multiplication plots); the traits (seed weight, seed colour, and cotyledon colour determined spectrophotometrically from seed meals in absence of testa) were analysed by interval mapping (IM-QTL). For seed weight analysis in these RILs, data were also collected for the $F_7$ and $F_{10}$ seeds from SSD plants grown in the glasshouse.

Significant QTL were determined for all traits, with good consistency across experiments. The QTL determined for mean seed weight in five contrasting experiments are listed in Table 2.1. The QTL on LGI and LGV were evident across five experiments, while that on LGIV was detected in three experiments and was overall of lower significance. Since these and the QTL determined for seed and cotyledon colour have been determined for seeds from plants grown under very contrasting conditions, it is likely that they will prove to be robust; verification will depend on the data acquired for the 2010 plots.

Genetic map construction for combining populations

Evaluation of the relatedness of the three parents from the combining crosses in 1.1 above identified SSAP markers, based on PDR1 retrotransposon insertion sites, which were expected to segregate in the crosses and provide anchor markers for the seven linkage groups. $F_6$ DNA from 180 - 200 lines from every cross is being analysed, and maps are under construction as described previously.

$F_6$ DNA from the three populations, BE, BK and EK had been extracted previously and stored in six 96 well plates. Fluorescent SSAP markers using all sixteen possible selective primers had been used previously to screen the three parent lines for potential polymorphisms. Comparison of Brutus and Kahuna showed the highest number of polymorphic markers (124), followed by Enigma and Kahuna (110) and finally Brutus and Enigma (72). Ten primer combinations (of the possible sixteen above) were used on the two DNA plates for the BK population, which gave 88 markers from a potential 100. The marker data were analysed using Joinmap to give 17 small groups of linked markers with 4 unlinked markers. By comparison with the linkage maps that had been assembled for wide crosses in pea, using SSAP markers, the 17 BK groups were assigned to the seven pea linkage groups.

Twelve primer combinations were used on the EK population which gave 90 markers from a potential 100. The marker data were analysed using Joinmap to give 15 small groups of linked markers with 4 unlinked markers. By comparison with other linkage maps that utilised these markers (as above), the 15 EK groups were assigned to the seven pea linkage groups.

For the Brutus x Enigma population with the lowest number of potential markers, some SSAP markers have been scored and investigations are underway regarding the use of other types of markers such as microsatellites to provide sufficient polymorphic markers to build a map.

An excel spreadsheet giving the map markers and order for the combining populations is available on request. The current state of BK and EK maps derived from the combining RILs is shown in Appendix Figure 1, where the seven linkage groups are aligned based on orders determined for other crosses. Here the markers determined for BE and BK RILs are colour- coded according to the number of crosses in which they occur (Appendix Figure 1).

**Average seed weight QTL determined for MxS RILs**

| Population | Linkage group | LG locus with highest LOD value | Max. LOD value | Variation explained (%) | Additive genetic effect |
|---|---|---|---|---|---|
| **F7 (glasshouse)** | LG I | CT_824 | 7.99 | 21.5 | 0.026944* |
| | LG IV | Tps1/194- | 3.15 | 9.2 | 0.0135009* |
| | LG V | Tps1/237- | 4.9 | 13.9 | 0.0166986* |
| **F8 (glasshouse)** | LG I | CT_824 | 10.12 | 26.4 | 0.0221615* |
| | LG IV | Tps1/168 | 2.31 | 6.9 | 0.0113349* |
| | LG V | Tps1/237- | 2.93 | 8.6 | 0.0126951* |
| **F10 (glasshouse)** | LG I | CT_824 | 4.68 | 14.1 | 0.0224186* |
| | LG V | Tps1/237- | 2.77 | 8.7 | 0.0176128* |
| | LG VII | Tps1/22 | 2.5 | 7.8 | 0.016664* |
| **F8 (field)** | LG I | CT_824 | 6.11 | 21.7 | 0.0147109* |
| | LG IV | Tps1/241 | 3.53 | 13.4 | 0.011557* |
| | LG V | Tps1/237- | 3.32 | 12.6 | 0.0110892* |
| **F9 (field)** | LG I | CT_824 | 6.45 | 18.6 | 0.0118228* |
| | LG V | Tps1/237- | 6.1 | 18.3 | 0.0117434* |

* indicates that the allele derived from the parent M increases the value of the trait

*Table 2.1: Map location, percentage of phenotypic variation accounted for, additive effects and detected QTL for average seed weight from five experiments using MxS RILs.*

## 2.2 To provide for genetic marker development in pea

Marker development in pea follows two general strategies: a) The development of gene specific markers in order to anchor the pea genetic map against the genome sequence of related legumes, especially *Medicago truncatula*, soybean and *Lotus japonicus* and b) the development of multiplex markers that are highly polymorphic in pea and relate the genetic maps of different crosses.

2.2a) Gene specific markers

Genetic mapping in the RIL populations JI281xJI399, JI15xJI399, JI15xJI1194 and JI2822xJI2202 has included gene specific markers. These markers have the potential to be used for the alignment of the pea genetic map with sequenced genomes. For the RIL population derived from JI281xJI399, the markers used are of two types. The first of these correspond to amplicons mapped in a subset of 16 RILs (in collaboration with G. Kiss' laboratory at Godollo, in the Grain Legumes Integrated Project). These fall into two classes: those designed with reference to pea sequences and those designed with reference to *M. truncatula* sequences. This complicates the identification of orthologous loci, because the pea genomic sequence is not always known. The second class of sequence corresponds to pea sequences used as RFLP markers or for PCR markers and that have been mapped in the full population. In this class of markers it is possible to identify orthologous sequences in pea and *M. truncatula.* For these sequences the CVIT BLAST tool can be used to identity the location of *M. truncatula* sequences and to rank the corresponding identity, for example either as most similar pairs or as an identity with a particular degree of relatedness. The result of such an analysis is illustrated below.

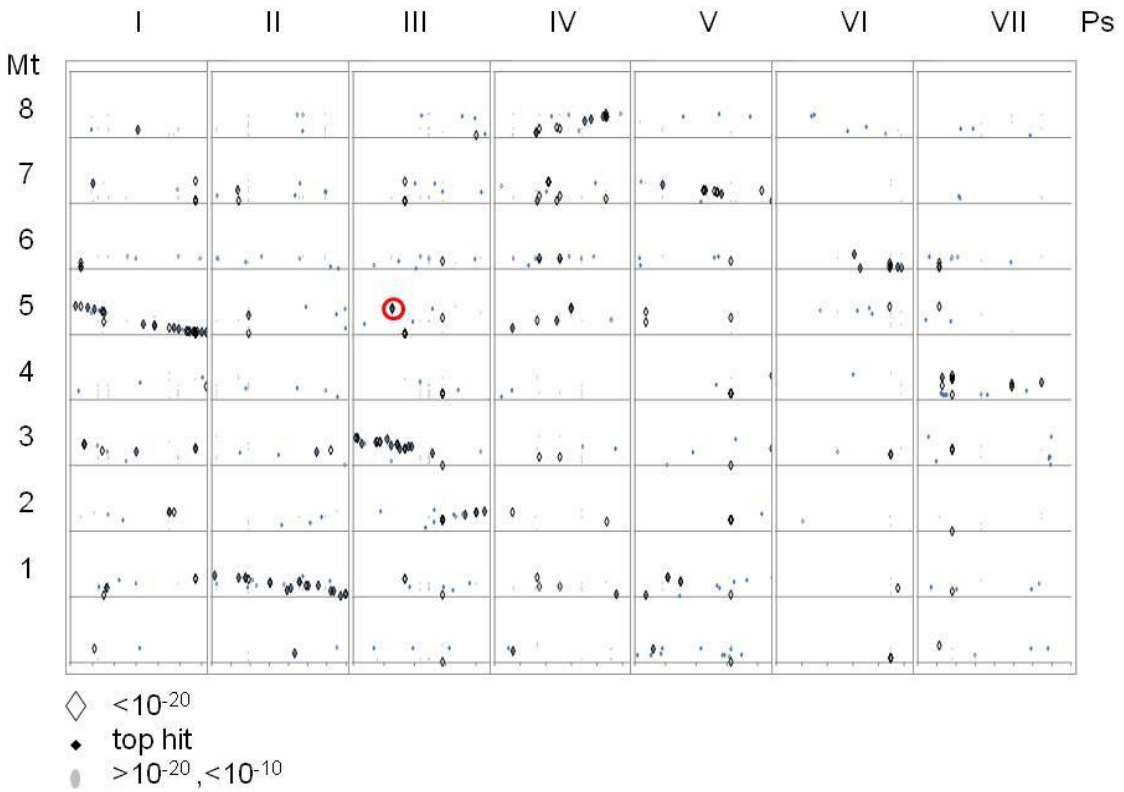



*Figure 2.1: Alignment of the pea genetic map with the M. truncatula genome sequence assembly. The x-axis corresponds to the position of the genetic locus for a given pea sequence on the pea linkage map and the y-axis to the position of a corresponding sequence in the M. truncatula genome sequence. The ranking of the BLAST score is indicated by one of three symbols.*

The comparison of the pea genetic map and the *M. truncatula* genome sequence corresponds well to what was previously known about the alignment of these two genomes, but the resolution of this map is higher and supports evidence for some previously identified small scale rearrangements, such as the small cluster of BLAST hits scoring $<10^{-20}$ and 'top hits' on *M. truncatula* chromosome 5 but mapping to pea linkage group III (ringed in red). The combination of this data set with previous map data will be undertaken with the aim of preparing a publication in 2011/12.

The genetic map of the inter-subspecific cross JI2822 x JI2202 (*P. sativum* x *P. abyssinicum*) has the potential to provide a high resolution map because of the extent of genomic differentiation between *P. sativum* and *P. abyssinicum*. This population has been studied for several years, but it has been problematic to construct a genetic map. The development of the Threadmapper programme (Cheema et al. 2010) has facilitated the construction and visualisation of the corresponding genetic map. See Appendix Figure 2 for a Threadmapper display of a translocation breakpoint in the genetic map of JI2822 x JI2202.

The Threadmapper software permits analysis and display of the distance matrix of all possible pairs of markers. This is presented below for the map of the *P. sativium x P. abyssinicum* RIL population. Here the markers are in order along the map and the fraction of lines that are recombinant between markers x and y are plotted in the x y plane and colour coded according to the value; blue is a low value and red a high value.

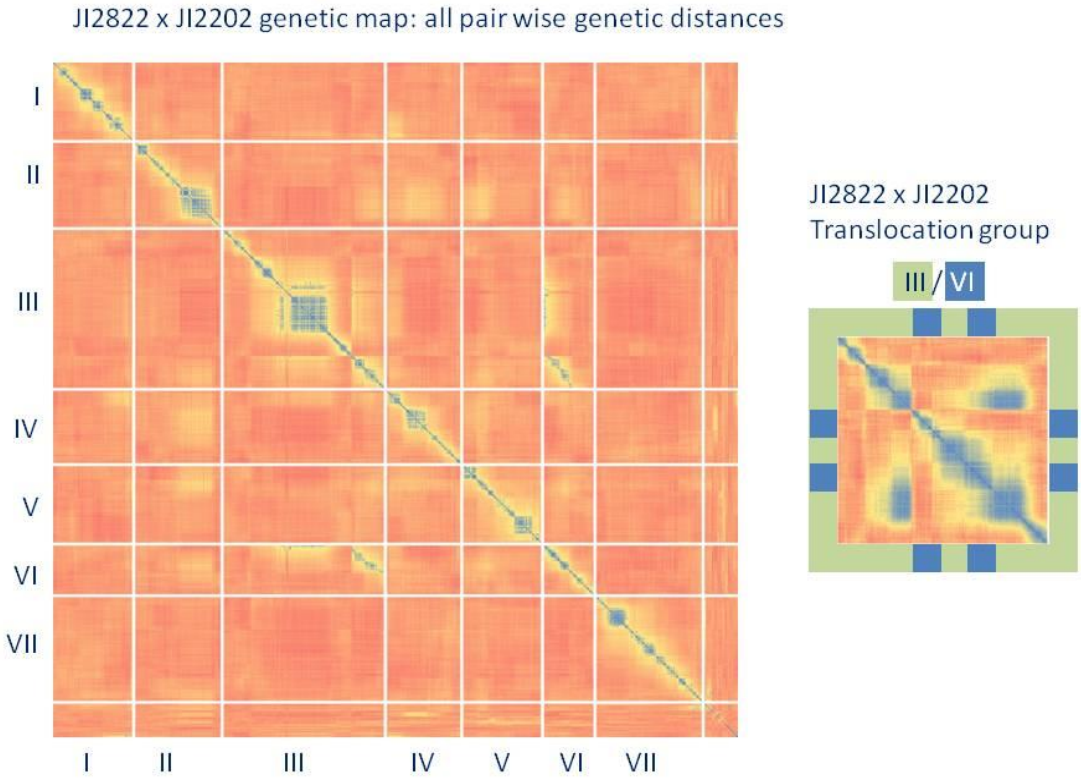



*Figure 2.2: Distance matrix corresponding to all pairwise inter-marker distances in the genetic map for JI2822xJI2202 RILs*

The expectation of a genetic map is to see a diagonal blue line surrounded by yellow. To a large degree this is what is seen, with the exception of the intersection of linkage groups III and VI. The panel to the right combines the linkage group III and linkage group VI derived markers in this composite group, with the segments of linkage group III and linkage group VI markers identified by the border colour.

Thus we have largely resolved the genetic map structure of this wide cross population. The availability of this RIL population for future genetic mapping will assist in mapping markers where the diversity within *P. sativum* is relatively low.

2.2b) Multiplex markers

There has been extensive use of insertion site polymorphism of the pea Ty1-copia like retrotransposon, PDR1, both for genetic diversity studies and for genetic mapping. This marker is the basis of the genetic mapping discussed in section 2.1 above. Comparisons of the order of these markers across seven *Pisum* crosses is underway not only to validate linkage information and indicate regions where problems occur, but also to help in the identification of genes and markers within the marker-free zones of the PCGIN maps, especially those derived from the combining lines (BE, BK and EK).

**2.3 To provide for genetic marker development in faba bean**

Since the last report, we have applied the set of 75 KASPar SNP assays to a further generation of SSD to confirm that no undesired outcrossing took place and that heterozygosity decreased by approximately 50% at each selfing.

TGAC (The Genome Analysis Centre) have supplied NIAB with 454 sequence generated from RNA from 7 day old root and shoot material from two faba bean lines. Following assembly, ~14, 000 contigs were found to be common to both lines with an average of 3 SNPs/Kbp. In the absence of a *Vicia faba* genomic sequence, SNPs have been ordered by identifying orthologous sequences in *Medicago truncatula*, which has synteny with *Vicia faba* (Ellwood et al., 2008, BMC Genomics 9: 380). A further 200 gene-based SNP assay set is in development and will be available for use within the PCGIN project.

**2.4 To provide updates on genetic marker development in lupin**
The most recent version of the lupin genetic map is: Aligning a New Reference Genetic Map of Lupinus angustifolius with the Genome Sequence of the Model Legume, Lotus japonicus, Nelson et al (2010) published online by DNA Research at
http://dnaresearch.oxfordjournals.org/cgi/content/abstract/dsq001v1.
This study was supported by the EU FP6 Integrated project 'Grain Legumes'.

**3. Genetic resources**

**3.1 To provide novel genetic resources for pea**
**3.1.1 To obtain novel mutants associated with quality traits**

The gene encoding PaO (pheophorbide *a* oxygenase) had been targetted for the identification of novel mutants. Several *PaO* mutants were obtained by TILLING, and these were phenotyped and genotyped. One mutant family gave rise to a seed with green cotyledons, indicative of a block in the chlorophyll degradation pathway. The mutation affected the predicted overall charge of the region near the mononuclear iron-binding site and was evident in one seed only. This seed did not germinate and the corresponding heterozygotes were sterile. Seeds from multiplications of earlier generations will be sown in case the mutation may be recovered. Additional genes from the pathway have been targeted for mapping (see earlier) and as candidates for the isolation of further mutants by TILLING.

**3.1.2 To obtain novel mutants associated with performance traits**
The use of the FN population derived from JI2822 has been a major objective within PCGIN. In previous reports and publications we have undertaken a gel-based AFLP screen in order to identify sequences lost in FN deletion mutants. Previous analysis of the *arthritic* mutant identified several band differences between wild type and mutant lines (PCGIN I report).

*Figure 3.1: The arthritic mutant (*art*) in comparison with wild type*

A FN deletion mutant allele of arthritic is shown in comparison to its progenitor wild type. The swollen node is clearly seen with the expanded area of pigmentation in the axil ring. Note the additional vertical stripe of colour antipodal to the insertion of the petiole.

These multiple differences were unexpected and may have represented DNA methylation differences (at the PstI site) or unsuspected heterogeneity in the JI2822 progenitor material. Accordingly we decided to try to obtain additional information on this mutant using next-generation sequencing of the AFLP fragments. These amplicons were submitted to TGAC at the end of the previous reporting year. The sequence runs were undertaken late in 2010 and preliminary information is summarised below.

**3.1.3 To generate NILs for QTL identified by areas 1 & 2**
This activity will follow on from the QTL analyses in 2.1, and selection of genotypes from RILs.

### 3.1.4 To analyse fast neutron deletions affecting plant architecture

The gene *Art* (*arthritic*) was selected as a potential target of interest to PCGIN because this affected stem architecture, with swelling at each node. The feasibility of using next generation sequencing methods to characterise differences between the transcriptomes of mutant and wild type sister lineages is being investigated and experiments are ongoing with TGAC. The informatic analysis of these sequences is planned for 2011/12.

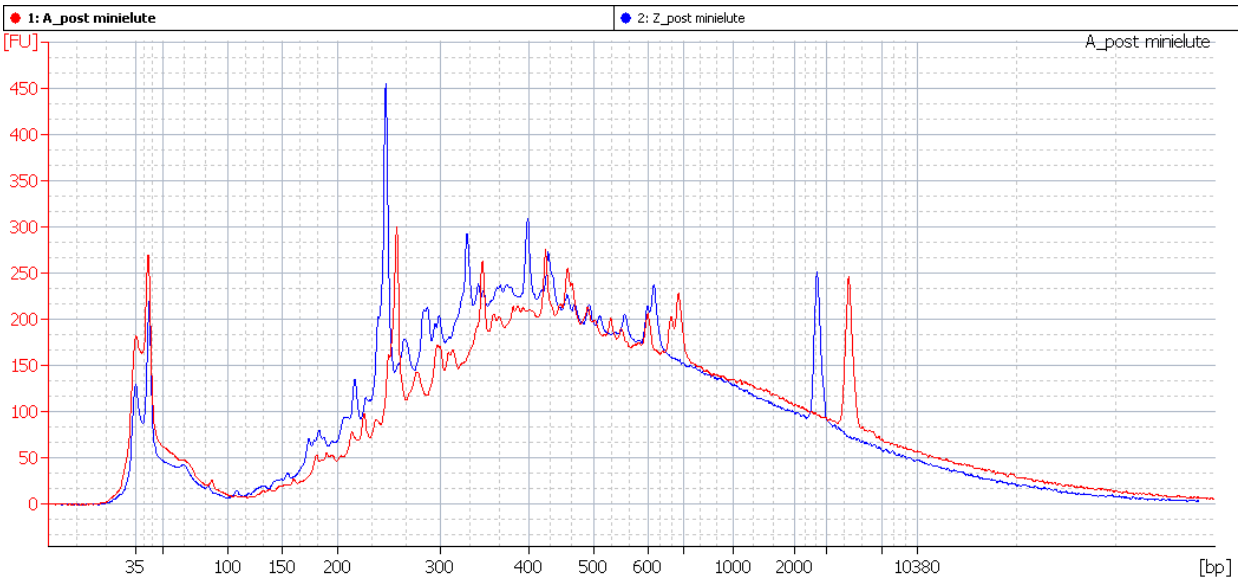



*Figure 3.2: Frequency distribution of sequence run lengths. Blue: JI2822, Red: art1.*

## 3.2 To expand the genetic resources for faba bean in UK

### 3.2.1 To establish faba bean inbred lines

A rolling programme of advancement through generations of inbreeding is now well-established, with up to 20 new lines added each year and 20 'finished' F6 lines entering multiplication for field-scale phenotyping (see Appendix Figure 3). The first F6 lines are due to be harvested in June 2011 and the most recently added set of lines (19 UK broad bean lines bringing the total number to 108) will be at F6 in Winter 2013. In each generation, DNA from a leaf sample of each plant is genotyped with the 75-SNP set of KASPar assays to assess heterozygosity.

### 3.2.2 To provide phenotypic descriptors of faba bean lines and accessions

Phenotypic characters have previously been collected from lines in SSD and screens of accessions for rust and downy mildew resistance have been carried out in controlled environments. Promising material from the latter has been included in SSD. Additional screens for chocolate spot (*Botrytis fabae*) resistance were carried out in 2010. Single leaflet pairs at the third node were inoculated in controlled environment room with a droplet of inoculum. Some lines exhibited rapid disease development and "aggressive" chocolate spot lesions (Figure 3.3a) with spread to leaves distant from the point of inoculation. Other lines remained with limited spotting only (Figure 3.3b) and no spread to other leaflets. Two of these lines, NV 175 and NV 651 are continuing in SSD. A resistant phenotype which confines chocolate spot to the non-aggressive stage may prove valuable under UK conditions by reducing the rate of epidemic development.

**A**

*Figure 3.3 Responses of faba bean leaves to inoculation with Botrytis fabae; a, left –aggressive ; b, right – non-aggressive*

**3.2.3 To establish mapping populations for faba bean**
This activity is due to commence in 2011.

**4. Management & communication**

**4.1 To manage PCGIN in a responsive manner**
It had been suggested that a stakeholders' meeting be combined with the PGRO open day in June 2010 and this was a successful formula, reaching a wide audience. A presentation on PCGIN activities was followed by inspection of the PCGIN multiplication plots (EK population), and generated much interest from the press. This arrangement will be repeated in 2011.

**4.1.1 To establish related programmes of work**
A LINK project (Quality Determinants in Pea Seeds, QDiPS) began in 2010 with joint funding from BBSRC and Defra. This provides a consortium with industry that is distinct but complementary to the PCGIN stakeholder group.

Discussions with industry proceeded during 2010 related to the two TSB calls for crop protection and for sustainable protein production. The first of these has culminated in an industry-led project involving NIAB, building on the PCGIN resources. The next proposals will be submitted in 2011.

**4.2 To integrate PCGIN with international activities**

PCGIN research was well-represented at the IFLRC V & AEP VII meeting, Antalya, Turkey, 2010 (presentations by N. Ellis, C. Domoney and a poster contribution). This also provided the opportunity for collaborative discussions with several labs, including that at University of Saskatchewan. D. O'Sullivan (NIAB) visited ICARDA, Syria and agricultural research institutes in Egypt funded by a BBSRC ISIS award to discuss achievements within PCGIN and to develop contacts for future international collaboration. A project based on addressing key problems of faba bean in sub-Saharan Africa has been submitted to the recent BBSRC SCPRID call. Further links with Germany, Egypt and Canada have been developed by D. O'Sullivan in order to maximize the synergies beween various national faba bean initiatives.

**4.3 To disseminate and publish results**
PCGIN has continued to be represented at various public events, where public engagement and dialogue is promoted. In 2010, these events included PGRO open days (pulse and vining) and Limagrain open day.

See section 11.

## Amendments to project

9. Are the current scientific objectives appropriate for the remainder of the project? ..... **YES** ☒ **NO** ☐
If **NO**, explain the reasons for any change giving the financial, staff and time implications.
**Contractors cannot alter scientific objectives without the agreement of the Defra Project Manager.**

## Progress in relation to targets

10. (a) List the agreed milestones for the year/period under report as set out in the contract or any agreed contract variation.
It is the responsibility of the contractor to **check fully that all milestones have been met** and to provide a detailed explanation when they have not been achieved.

| Milestone | | Target date | Milestones met | |
|---|---|---|---|---|
| Number | Title | | In full | On time |
| M9 | Develop F6 based genetic maps for pea RIL populations | Month 22 | Yes | Yes |
| M13 | Validate marker toolkit for faba bean and determine genetic diversity of EU inbred material | Month 22 | Yes | Yes |
| M15 | Discuss strategy with management group for the collection and testing of novel mutants and variants available for pea | Month 16 | Yes | Yes |
| M17 | Identify candidates for art mutation in pea | Month 14 | Yes | Yes |
| M18 | Establish methods for bean downy mildew and rust resistance phenotyping, and initiate culture collection | Month 14 | Yes | Yes |
| M24 | Meet with stakeholders' as update on research activities | Month 22 | Yes | Yes |

(b) Do the remaining milestones look realistic? ..... **YES** ☒ **NO** ☐
If you have answered **NO**, please provide an explanation.

## Publications and other outputs

11. (a) Please give details of any outputs, e.g. published papers/presentations, meetings attended during this reporting period.

**Presentations:**

**JIC:**

Invited keynote lectures at IFLRC V & AEP VII meeting, Antalya, Turkey, 2010
Invited chair of session at IFLRC V & AEP VII meeting, Antalya, Turkey, 2010
Invited presentation of PCGIN science at PGRO open day, July 2010
Metabolic Biology departmental seminar, JIC, September 2010
Presentations to various industries: Wherry, Limagrain, TSB for project development, Autumn 2010

**Publications:**

**JIC:**

CHEEMA, J., ELLIS, T. H. N. and DICKS, J. (2010) THREaD Mapper Studio: a novel, visual web server for the estimation of genetic linkage maps. Nucleic Acids Research 38, W188-W193

CHINOY, C., WELHAM, T., TURNER, L. MOREAU, C. and DOMONEY, C. (2011) The genetic control of seed quality traits: effects of allelic variation at the *Tri* and *Vc-2* genetic loci in *Pisum sativum* L. Euphytica DOI 10.1007/s10681-011-0363-8

CLEMENTE, A., MORENO, F.J., MARÍN-MANZANO, M.C., JIMÉNEZ, E. and DOMONEY, C. (2010) The cytotoxic effect of Bowman-Birk isoinhibitors**,** IBB1 and IBBD2, from soybean (*Glycine max*) on HT29 human colorectal cancer cells is related to their intrinsic ability to inhibit serine proteases. Molecular Nutrition and Food Research *54*, 396-405

CLEMENTE, A., SONNANTE, G. and DOMONEY, C. (2011) Nutritional implications of Bowman-Birk inhibitors from legumes on human gastrointestinal health: current status and perspectives. Current Protein & Peptide Science (in press)

CLEMENTE, A., MARÍN-MANZANO, M. C., JIMÉNEZ, E. SONNANTE, G., MORENO, F. J., RUBIO, L. A. and DOMONEY, C. (2010) The cytotoxic effects of Bowman-Birk proteins from legumes on colon cancer cells are related to their ability to inhibit serine proteases. In: Legumes for global health, Legume crops and products for food, feed and environmental benefits. Fifth International Food Legumes Research Conference (IFLRC V) & Seventh European Conference on Grain Legumes (AEP VII), Antalya, Turkey, p. 22

CLEMENTE, A., MARÍN-MANZANO, M. C., JIMÉNEZ, E., MORENO, F. J., RUBIO, L. A. and DOMONEY, C. (2010) The anti-proliferative properties of Bowman-Birk proteins from legumes on colon cancer cells are related to their ability to inhibit trypsin- and chymotrypsin-like proteases. In: BIT Inaugural Symposium on Enzymes & Biocatalysts, Shanghai, China, p. 173

CLEMENTE, A., MARÍN-MANZANO, M. C. and DOMONEY, C. (2010) Exploiting natural variation in legume Bowman-Birk inhibitors to dissect their potential role in human health-

promoting programmes. In: 8th Canadian Pulse Research Workshop, Calgary, Alberta, Canada

DOMONEY, C., CHINOY, C., PILLINGER, W., HASENKOPF, K., WILD, F., WARKENTIN, T., CLEMENTE, A. and CHARLTON, A. (2010) The genetic control of seed quality traits that impact on food and feed uses of pea. In: Legumes for global health, Legume crops and products for food, feed and environmental benefits. (Keynote) Fifth International Food Legumes Research Conference (IFLRC V) & Seventh European Conference on Grain Legumes (AEP VII), Antalya, Turkey, p. 53

ELLIS, N. (2011) Germplasm resources in legumes. Plant Genetic Resources: Characterization and Utilization *9*, 1-3

HELLENS, R. P., MOREAU, C., LIN-WANG, K., SCHWINN, K. E., THOMSON, S. J., FIERS, M. W., FREW, T. J., MURRAY, S. R., HOFER, J. M., JACOBS, J. M., DAVIES, K. M., ALLAN, A. C., BENDAHMANE, A., COYNE, C. J., TIMMERMAN-VAUGHAN, G. M. and ELLIS, T. H. (2010) Identification of Mendel's white flower character. PLoS One *5*, e13230

KNOX, M., DOMONEY, C., COYNE, C., AMBROSE, M. and ELLIS, N. (2010) Relationship patterns in *Pisum* assessed with a range of molecular marker types. In: Legumes for global health, Legume crops and products for food, feed and environmental benefits. Fifth International Food Legumes Research Conference (IFLRC V) & Seventh European Conference on Grain Legumes (AEP VII), Antalya, Turkey, p. 159

JING, R., VERSHININ, A., GRZEBYTA, J., SHAW, P., SMÝKAL, P., MARSHALL, D., AMBROSE, M. J., ELLIS, T. H. and FLAVELL, A. J. (2010) The genetic diversity and evolution of field pea (Pisum) studied by high throughput retrotransposon based insertion polymorphism (RBIP) marker analysis. BMC Evolutionary Biology *10*, 44

RISPAIL, N., KALÓ, P., KISS, G. B., ELLIS, T. H. N., GALLARDO, K., THOMPSON, R. D., PRATS, E., LARRAINZAR, E., LADRERA, R., GONZÁLEZ, E. M., ARRESE-IGOR, C., FERGUSON, B., GRESSHOFF, P. M. and RUBIALES, D. (2010) Model legumes contribute to faba bean breeding. Field Crops Research *115*, 253-269

RUBIALES, D., AMBROSE, M. J., DOMONEY, C. and BURSTIN, J. (2011) Pea (*Pisum sativum* L.). Chapter 1, In: Genetics, Genomics and Breeding in Crop Plants: Cool Season Food Legumes (Ed. Kole, C.). Science Pubs Inc., New Hampshire, Jersey, Plymouth (in press).

SMYKAL, P., KENICER, G., FLAVELL, A.J., CORANDER, J., KOSTERIN, O, REDDEN, R.J., FORD, R., COYNE, C.J., MAXTED, N., AMBROSE, M.J. and ELLIS, T.H.N. (2011) Phylogeny, phylogeography and genetic diversity of the Pisum genus. Plant Genetic Resources: Characterization and Utilization *9*: 4-18

**NIAB:**
SILLERO, J.C., VILLEGAS-FERNÁNDEZ, A., THOMAS, J.E., ROJAS-MOLINA, M., EMERAN, A., FERNÁNDEZ-APARICIO, A. and RUBIALES D (2010) Faba bean breeding for disease resistance. Field Crops Research *115:* 297-307

STODDARD, F., NICHOLAS, A., THOMAS, J.E., VILLEGAS-FERNANDEZ, A. (2010) Integrated pest management in faba bean. Field Crops Research *115:* 308-318

(b) Have opportunities for exploiting Intellectual Property arising out of this work been identified? ..... **YES** ☐ **NO** ☒

If **YES**, please give details.

(c) Has any other action been taken to initiate Knowledge Transfer?..... **YES** ☒ **NO** ☐
If **YES**, please give details.

Discussions with industry in relation to new project proposals have taken place.

Will Pillinger, Limagrain, completed his M.Sc. studies in 2010 at JIC. Some of the lines used by him, together with some of the PCGIN and mapping RILs, have been incorporated into his breeding programme.

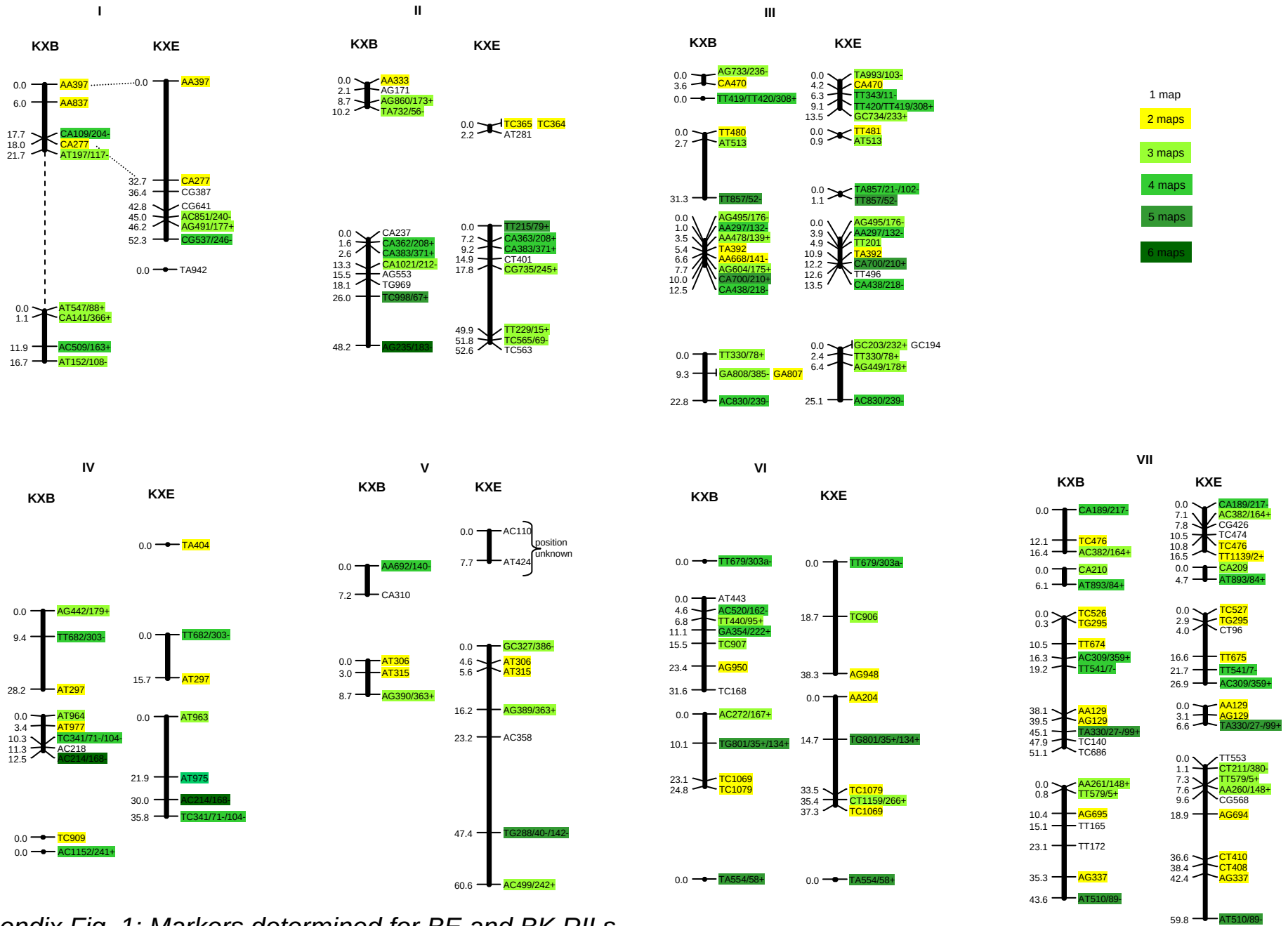
## Future work

12. Please comment briefly on any new scientific opportunities which may arise from the project.

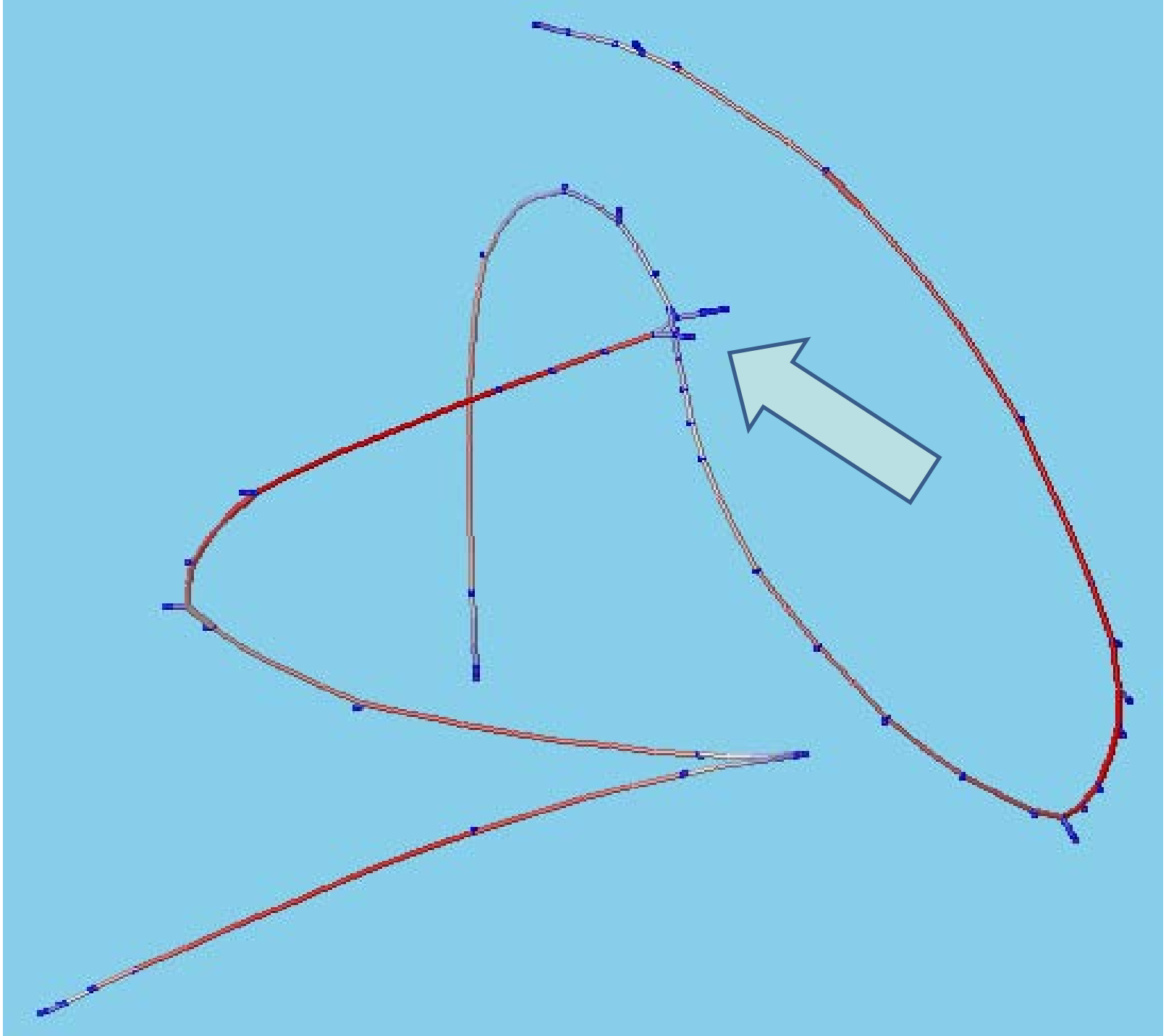
## Declaration

13. I declare that the information I have given is correct to the best of my knowledge and belief.

| | | | |
|---|---|---|---|
| Name | Dr Claire Domoney | Date | 11/4/11 |
| Position held | Project Leader, Department of Metabolic Biology, John Innes Centre | | |




*Appendix Fig. 1: Markers determined for BE and BK RILs*



*Appendix Fig. 2: Threadmapper display of a translocation breakpoint in the genetic map of the JI2822xJI2202 cross (arrow), where the other crossing point is resolved in the third dimension of the display.*

| | | Generation | | | | | |
|---|---|---|---|---|---|---|---|
| NV number | Donor Reference | S1 | S2 | S3 | S4 | S5 | S6 |
| 1 NV103-1 | Ig11903 | | | | | | S6 |
| 2 NV129-2 | Ig12137 | | | | | | S6 |
| 3 NV150-2 | Ig12613 | | | | | | S6 |
| 4 NV271-1 | Ig14189 | | | | | | S6 |
| 5 NV594-2 | Ig132660 | | | | | | S6 |
| 6 NV134-1 | Ig12159 | | | | | S5 | |
| 7 NV138-1 | Ig12263 | | | | | S5 | |
| 8 NV153-1 | Ig12658 | | | | | S5 | |
| 9 NV163-2 | Ig12747 | | | | | S5 | |
| 10 NV175-1 | Ig13004 | | | | | S5 | |
| 11 NV20-2 | Ig11290 | | | | | S5 | |
| 12 NV275-2 | Ig14197 | | | | | S5 | |
| 13 NV357-1 | Ig72495 | | | | | S5 | |
| 14 NV474-1 | Ig124126 | | | | | S5 | |
| 15 NV565-2 | Ig130596 | | | | | S5 | |
| 16 NV590-1 | Ig131693 | | | | | S5 | |
| 17 NV648-1 | BPL10 | | | | | S5 | |
| 18 NV649-1 | BPL11 | | | | | S5 | |
| NV649-2 | BPL11 | | | | | S5 | |
| 19 NV652-2 | BPL23 | | | | | S5 | |
| 20 NV653-2 | BPL27 | | | | | S5 | |
| 21 NV655-2 | BPL63 | | | | | S5 | |
| 22 NV163-1 | Ig12747 | | | | S4 | | |
| 23 NV20-1 | Ig11290 | | | | S4 | | |
| 24 NV266-6 | Ig14096 | | | | S4 | | |
| 25 NV336-1 | Ig72423 | | | | S4 | | |
| 26 NV474-2 | Ig124126 | | | | S4 | | |
| 27 NV490-3 | Ig124213 | | | | S4 | | |
| 28 NV511-1 | Ig124300 | | | | S4 | | |
| 29 NV512-1 | Ig124301 | | | | S4 | | |
| 30 NV574-1 | Ig130638 | | | | S4 | | |
| 31 NV589-2 | Ig130734 | | | | S4 | | |
| 32 NV604-1 | Borington Bulk | | | | S4 | | |
| 33 NV605-4 | V219 | | | | S4 | | |
| 34 NV606-3 | V220 | | | | S4 | | |
| 36 NV620-1 | Vf172 | | | | S4 | | |
| 37 NV638-2 | GLV45 | | | | S4 | | |
| 38 NV639-1 | Hedin/2 | | | | S4 | | |
| 39 NV640-3 | Maris Bead | | | | S4 | | |
| 40 NV641-4 | Fuego | | | | S4 | | |
| 41 NV642-1 | Granit | | | | S4 | | |
| 42 NV644-1 | Kasztelan | | | | S4 | | |
| 43 NV646-3 | Ig101760 | | | | S4 | | |
| NV648-2 | BPL10 | | | | S4 | | |
| NV648-3 | BPL10 | | | | S4 | | |
| NV649-3 | BPL11 | | | | S4 | | |
| 44 NV650-4 | BPL12 | | | | S4 | | |
| 45 NV651-3 | BPL21 | | | | S4 | | |
| NV652-3 | BPL23 | | | | S4 | | |
| 46 NV654-1 | BPL40 | | | | S4 | | |
| NV654-4 | BPL40 | | | | S4 | | |
| 47 NV656-3 | BPL183 | | | | S4 | | |
| 48 NV658-2 | CGN07715 | | | | S4 | | |
| 49 NV660-1 | FAB4000 | | | | S4 | | |
| 50 NV73-6 | Ig11656 | | | | S4 | | |
| 51 NV82-5 | Ig11695 | | | | S4 | | |
| 52 NV100-5 | Ig11749 | | | S3 | | | |
| 53 NV106-5 | Ig11953 | | | S3 | | | |
| 54 NV1-5 | Ig11195 | | | S3 | | | |
| 55 NV155-5 | Ig12684 | | | S3 | | | |
| 56 NV169-1 | Ig12761 | | | S3 | | | |
| 57 NV211-5 | Ig13822 | | | S3 | | | |
| 58 NV242-5 | Ig13948 | | | S3 | | | |
| 59 NV248-1 | Ig13978 | | | S3 | | | |
| 60 NV2-5 | Ig11197 | | | S3 | | | |
| 61 NV266-4 | Ig14096 | | | S3 | | | |
| 62 NV27-1 | Ig11312 | | | S3 | | | |
| 63 NV284-5 | Ig70718 | | | S3 | | | |
| 64 NV28-5 | Ig11313 | | | S3 | | | |
| 65 NV290-5 | Ig70723 | | | S3 | | | |
| 66 NV293-5 | Ig70726 | | | S3 | | | |
| 67 NV38-5 | Ig11398 | | | S3 | | | |
| 68 NV422-1 | Ig115182 | | | S3 | | | |
| 69 NV424-5 | Ig115227 | | | S3 | | | |
| 70 NV53-5 | Ig11531 | | | S3 | | | |
| 71 NV596-2 | Ig132813 | | | S3 | | | |
| 73 NV643-3 | Albus | | | S3 | | | |
| 74 NV653-3 | BPL27 | | | S3 | | | |
| 75 NV662-1 | Vf136 | | | S3 | | | |
| 76 NV672-1 | Betty | | | S3 | | | |
| 77 NV673-2 | Fury | | | S3 | | | |
| 78 NV674-3 | Memphis | | | S3 | | | |
| 79 NV675-3 | Pyramid | | | S3 | | | |
| 80 NV79-7 | Ig11687 | | | S3 | | | |
| 81 NV13-1 | Ig11276 | | S2 | | | | |
| 82 NV133-5 | Ig12158 | | S2 | | | | |
| 83 NV31-1 | Ig11327 | | S2 | | | | |
| 84 NV318-1 | Ig72355 | | S2 | | | | |
| 85 NV576-1 | Ig130674 | | S2 | | | | |
| 86 NV661-1 | Vf6 | | S2 | | | | |
| 87 NV671-6 | Atlas | | S2 | | | | |
| 88 NV676-3 | Tattoo | | S2 | | | | |
| 89 NV682-1 | Bunyard exhibition | | S2 | | | | |
| 90 NV692-1 | Violetta | S1 | | | | | |
| 91 NV693-1 | Red Epicure | S1 | | | | | |
| 92 NV694-1 | Karmazyn | S1 | | | | | |
| 93 NV695-1 | Listra | S1 | | | | | |
| 94 NV696-1 | Statissa | S1 | | | | | |
| 95 NV697-1 | Witkiem Manita | S1 | | | | | |
| 96 NV698-1 | Jubilee Hysor | S1 | | | | | |
| 97 NV699-1 | Express | S1 | | | | | |
| 98 NV700-1 | The Sutton | S1 | | | | | |
| 99 NV701-1 | Stereo | S1 | | | | | |
| 100 NV702-1 | Medes | S1 | | | | | |
| 101 NV703-1 | Witkiem | S1 | | | | | |
| 102 NV704-1 | Dreadnought | S1 | | | | | |
| 103 NV705-1 | Statissa | S1 | | | | | |
| 104 NV706-1 | Crimson Flowered | S1 | | | | | |
| 105 NV707-1 | Aquadulce Claudia | S1 | | | | | |
| 106 NV708-1 | Masterpiece Green Longpod | S1 | | | | | |
| 107 NV709-1 | Imperial Green Longpod | S1 | | | | | |
| 108 NV710-1 | Green Windsor | S1 | | | | | |