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E-mail: research.competitions@defra.gsi.gov.uk

Annual/Interim Project Report for Period February - October 2015

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Project details

1. Defra Project code: CH0103
2. Project title: Pulse Crop Genetic Improvement Network II (PCGIN II)
3. Defra Project Officer: Dr Giulia Cuccato
4. Name and address of contractor:
   John Innes Centre
   Norwich Research Park
   Colney
   Norwich
   Postcode NR4 7UH
5. Contractor's Project Manager: Dr Claire Domoney
6. Project: start date 01/02/15
   end date 31/01/17

Objectives

7. Please list the 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 Officer giving the date of amendment.

WP1: Maintenance & enlargement of the consortium

WP2: Trait biology & genetics in P. sativum and V. faba

WP3: Identification of novel beneficial variants for pea seed composition

WP4: Development of toolkits for breeding programmes

Summary of Progress

8. Please summarise, in layperson's terms, 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.

WP1: Maintenance & enlargement of the consortium

1.1 Platform that supports wide-ranging projects in UK, EU & world-wide (www.viciatoolbox.org; www.pcgin.org)

1.2 Maximise opportunities for UK legume crop improvement (collaborations: USASK, INRA, ICRISAT, ICARDA)

1.3 Disseminate results of scientific research and the means by which they may be exploited by industry

1.4 Establish industry-led forum to promote the uptake of resources (TSB, Agri-Tech; BBSRC LINK where prior support established)

PCGIN was re-launched with a kick-off meeting on March 30th at PGRO as a platform for the genetic improvement of pulse crops, to spark and incubate new associated projects with industry. This meeting demonstrated the strength of support that exists for UK pulse crops and the industries present reinforced their interest in participating in the network. All partners continue to have strong collaborations within EU and world-wide.

Dissemination activities have included exhibitions and posters at shows (Cereals 2015 - JIC, PGRO and NIAB; RNS AgriTech & schools activities; NIAB Open Day; NIAB Science Event, JIC Defra visits), talks and seminars nationally (RAU, CLA and RASE meetings, Government advisors and policy-makers, local groups) and internationally (EUCARPIA, Research in Plant Genetics), popular articles, radio (including Farming Today) and TV interviews, newspieces on websites associated with publications, and scientific publications.

The British Growers-led Pea & Bean Group has not met since the departure of James Hallett. Another industry leader is needed; noone has been available to take this role due to time commitments but it needs to be discussed again more widely. TSB/IUK projects continue to be well-represented.

WP2: Trait biology & genetics in P. sativum and V. faba

2.1 Field trials of genetically defined lines (yield & quality)

Field trial data for pea RILs:

A subset of lines was selected based on the variation shown in 2013 harvested plots for further evaluation to test yield stability across seasons. There was no significant yield difference between the selected lines at NIAB for 2014 harvest, though the highest yielding RIL, KB 201, and the highest yielding elite cultivar, Prophet, were common across the years (Table 1). The highest TSW in 2014 was seen in Kahuna, as expected, though there was significant variation for TSW in RILs with Kahuna parentage (lsd 30.3, $P<0.05$).

RIL/cultivar	t/ha 14	TSW 14	t/ha13
BE 028	3.08	214.53	2.97
BE 039	1.69	225.07	5.06
BE 043	2.37	243.07	4.79
BK 021	1.67	299.33	5.03
BK 071	1.88	230.00	4.94
BRUTUS	1.80	235.87	4.02
EB 173	2.25	209.20	4.98
ENIGMA	1.30	207.20	4.04
KAHUNA	1.56	342.53	4.50
KB 110	2.06	232.00	3.06
KB 201	3.33	275.47	4.95
PROPHET	2.94	269.47	5.08

Table 1: Summary data from NIAB trial of pea RILs, parents and elite cultivar, Prophet

Field trials of PCGIN RILs (KE/EK lines) at PGRO highlighted several RILs which were high-yielding and equivalent to Prophet. Three lines in particular performed well.

New faba bean genetic mapping resources have been created with the goal of identifying genetic markers, linked to resistance to downy mildew (Peronospora viciae f. sp. fabae, DM) and chocolate spot (Botrytis fabae, CS) diseases. Historic disease screening data was used to identify accessions from the NIAB germplasm collection with DM resistance, whereas an inoculated screen was implemented to identify sources of resistance for to B. fabae.

2.3 downy mildew resistance locus in pea

Gene-specific markers have been used to populate the region containing the DM resistance locus on the JI 15 x JI 1194 LGI; for several of these markers, the assays are 'breeder-friendly' CAPS or dCAPS but could be converted to other formats (KASPar). Two markers show tighter linkage than those in use by breeders currently, offering advantage in selections.

2.4 downy mildew resistance in faba bean

A single line from the NIAB germplasm collection, NV13, was identified previously to exhibit resistance towards multiple different isolates of DM (data not shown). A highly susceptible line, Memphis, was selected as the other parent for generating the mapping population. Inbred seed (S6) for each variety was sown out for crossing in a temperature controlled, isolated glasshouse. Parental lines were genotyped using 794 KASP markers, developed previously under PCGIN, to establish levels of homozygosity. Parental lines were crossed reciprocally, by hand, prior to anthesis, with the aim of producing 20 seed for each reciprocal cross (NV13 x Memphis/ Memphis x NV13). Twenty-one seeds were generated from the NV13 x Memphis and 19 seed for the Memphis x NV13). F1 progeny seed were harvested and sown out for genotyping and selfing. Tissue samples have been taken for subsequent DNA extraction and genotyping with 19 KASP markers, to ensure heterozygosity and successful crosses. F1s will be selfed and the F2 progeny genotyped (>200 lines) using a sub-set of KASP markers.

2.5 Ascochyta fabae resistance in faba bean

A single line, 29H, was previously identified to demonstrate resistance to Ascochyta fabae (data not shown). A segregating population developed from a cross between 29H and the cultivar Albus, has been used previously to identify QTL linked to stem nematode resistance, using marker resources developed under PCGIN (TSB). Hence, genotyping data is already available for these parental lines. The 29H x Albus cross is being reconstructed to provide sufficient lines for mapping in the F2:3. F1 seed will be harvested, selfed and genotyped as for the DM populations, for production of approximately 200 F2 lines. Resistance screening in the F3 generation will be conducted in growth rooms at the seedling stage in order to provide results before the end of the project.

2.6 chocolate spot resistance (Botrytis fabae) in faba bean

In a growth room experiment, 67 accessions of inbred faba bean (Vicia faba) were inoculated with a mix of six UK isolates of chocolate spot (Botrytis fabae) (data not shown). Based on these results, four lines were chosen as parents for the crossing programme (Table 2).

NIAB ID	Accession name	Disease rating, type
NV248-1/S7	ig13978	highly susceptible, spring bean
NV293-5/S8	ig70726	highly susceptible, spring bean
NV640-3/S9	Maris Bead	highly resistant, spring bean
NV651-3A1/S6	BPL21	highly resistant, spring bean

Table 2: Lines demonstrating resistance/susceptibility to B. fabae

The parental lines were grown in a bee-proof cage during summer. The lines were genotyped previously with a common set of 75 KASP markers to establish levels of homozygosity. Reciprocal crosses were performed manually, between resistant and susceptible lines, prior to anthesis (Table 3). This produced eight sets of F1 lines. The F1 lines are currently being grown for DNA extraction and subsequent genotyping with 19 KASP markers (identical to those used for other F1 genotyping). These lines will be selfed to produced >200 F2 lines for genotyping.

NIAB ID	Crossed with	CS population
NV640-3/S9	NV248-1/S7	1.1/1.2
NV640-3/S9	NV293-5/S8	2.1/2.2
NV651-3A1/S6	NV248-1/S7	3.1/3.2
NV651-3A1/S6	NV293-5/S8	4.1/4.2

Table 3: Crossing programme (CS) for lines resistant (left column) and susceptible (right column) to B. fabae

WP3: Identification of novel beneficial variants for pea seed composition

3.1 Seed colour stability

Crossing has proceeded with the aim of introgressing mutant alleles into common backgrounds to investigate and compare their phenotypes. Research on SGRL has revealed that this protein is essential for normal growth and development, likely as a consequence of its role in chlorophyll turnover of imprtance particularly under conditions of high light intensity. On the basis of the research carried out on its function, this gene is unlikley to contribute to colour loss from seeds; conversely, mutants of this gene are unlikley to contribute to seed colour stability.

A tentative map position has been assigned to the novel gene, GC, which is not alleleic to SGR.

3.3 Evaluation of mutations on seed composition and plant yield

The mutants relevant to the vining pea industry are being multiplied by Barfoots, who will assess these later in UK trials. For novel germplasm variants, including high-yielding RILs, sufficient seeds will be made available for plot trials in 2016 at NIAB and PGRO. The stakeholders have highlighted the control of pea moth as an emergent problem, likely to worsen with any withdrawal of appropriate chemicals. Based on this, the design of plot trials in 2016 will be discussed.

WP4: Development of toolkits for breeding programmes

4.1 Novel germplasm resources: utilisation of synteny & transcriptome resources

4.2 Faba bean genetics:

mutant populations

Bean mutant progress is well ahead of milestone target dates. Briefly, 1,100 each of EMS and X-ray lines were grown and are available for use next year within a plan to either advance these first 2,200 lines to M4 or grow out additional M3s.

A number of interesting and novel mutant phenotypes (disease lesion mimics, stem branching, flower colour and shape) were observed and individual plant threshing of M3 seed from 2,200 M2 plants is being completed currently. Each line is individually barcoded and the phenotypic records gathered through the season and at harvest will be associated with the barcode ID.

At flowering, Emily Bailes (PhD student with Prof. Beverley Glover at University of Cambridge) visited for two days and identified several flower shape/colour mutant lines with which she is interested in working.

Amendments to project

9. Are the current 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 objectives without the agreement of the Defra Project Officer.

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
1 & 2	First Stakeholder meeting; Field trial data for RILs	31/03/15	x	x
3	Refined genotype sets maximizing diversity	31/03/15	x	x
4	Existing trait datasets collated	31/07/15	x	x
5	New traits defined	31/08/15	x	x
6	Analysis of marker data	30/09/15	x	x
7	First Interim written report to Defra	31/10/15	x	x

(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.

BELL, A., MOREAU, C., CHINOY, C., SPANNER, R., DALMAIS, M., LE SIGNOR, C., BENDAHMANE, A., KLENELL, M. and DOMONEY, C. SGRL can regulate chlorophyll metabolism and contributes to normal plant growth and development in Pisum sativum L. Plant Mol. Biol., 2015

DOI: 10.1007/s11103-015-0372-4

CLEMENTE, A., ARQUES, M.C., DALMAIS, M., LE SIGNOR, C., CHINOY, C., OLIAS, R., RAYNER, T., ISAAC, P.G., LAWSON, D.M., BENDAHMANE, A. and DOMONEY, C. Eliminating anti-nutritional plant food proteins: the case of seed protease inhibitors in pea. PLoS ONE 10: e0134634 (1-24), 2015.

DOI: 10.1371/journal.pone.0134634

VAZ PATTO, M.C., AMAROWICZ, R., ARYEE, A.N.A., BOYE, J.I., CHUNG, H.-J., MARTÍN-CABREJAS, M.A. and DOMONEY, C. Achievements and challenges in improving the nutritional quality of food legumes. Crit. Rev. Plant Sci. 34, 105–143, 2015

DOI: 10.1080/07352689.2014.897907

WEBB, A., COTTAGE, A., WOOD, T., KHAMASSI, K., HOBBS,D., GOSTKIEWICZ, K., WHITE, M., KHAZAEI, H., ALI, M., STREET, D., DUC, G., STODDARD, F.L., MAALOUF, F., OGBONNAYA, F.C., LINK, W., THOMAS, J. AND O'SULLIVAN, D.M. A SNP-based consensus genetic map for synteny-based trait targeting in faba bean (Vicia faba L.). Plant Biotechnol. J., 2015

DOI: 10.1111/pbi.12371

DOMONEY, C., BELL, A., CHINOY, C., MOREAU, C., AMBROSE, M. and MAGUIRE, R. Mendel's green cotyledon gene, its variants and impact on phenotype. In: Research in Plant Genetics. Mendel's legacy: 150 years of the genius of genetics (Conference book, Mendel museum of Masaryk University, Brno), p. 69, 2015

WARKENTIN, T.D., SMYKAL, P., COYNE, C.J., WEEDEN, N., DOMONEY, C., BING, D.-J., LEONFORTE, A., XUXIAO, Z., DIXIT, G.P., BOROS, L., McPHEE, K.E., McGEE, R.J., BURSTIN, J. and ELLIS, T.H.N. Current status of pea breeding internationally. In: Research in Plant Genetics. Mendel's legacy: 150 years of the genius of genetics (Conference book, Mendel museum of Masaryk University, Brno), p. 77, 2015

WARKENTIN, T.D., SMYKAL, P., COYNE, C.J., WEEDEN, N., DOMONEY, C., BING, D.-J., LEONFORTE, A., XUXIAO, Z., DIXIT, G.P., BOROS, L., McPHEE, K.E., McGEE, R.J., BURSTIN, J. and ELLIS, T.H.N. Pea. In: Grain Legumes (Handbook of Plant Breeding, vol. 10. Ed. A.M. De Ron). Springer Science & Business Media, pp. 37-84, 2015.

DOI: 10.1007/978-1-4939-2797-5

DOMONEY, C., CHINOY, C., BELL, A., RAYNER, T., ISAAC, P.G., SPANNER, R., MAGUIRE, R. and CLEMENTE, A. Seed quality traits for a sustainable agriculture: understanding the genetic control of visual and compositional traits in pea. In: Proceedings of the International Symposium on Protein Crops, EUCARPIA (Ed. A. de Ron), 2015

CLEMENTE, A. and DOMONEY, C. Nutritional significance of Bowman-Birk inhibitors from legumes in food and feed. In: Proceedings of the International Symposium on Protein Crops, EUCARPIA (Ed. A. de Ron), 2015

MARÍN-MANZANO, M.C., ARQUES, M.C., OLÍAS, R., RUBIO, L.A., DOMONEY, C. and CLEMENTE, A. (2015) What makes Bowman-Birk inhibitors from legumes indigestible? Proceedings 4th International Conference Food Digestion, Naples, 17-19 March, p. 147, 2015

DOMONEY, C. Achievements and challenges in improving pea seed quality for food. In: Legume Perspectives: Legume Quality and Health (Ed. C. Vaz Patto). Journal of the International Legume Society. Abraka Dabra, Novi Sad, Serbia, 19-21, 2015

MEETINGS ATTENDED/PRESENTATIONS/POSTERS (see above, where proceeding papers)

SMITH, P. on behalf of DOMONEY, C. Genetic approaches to improved seed quality traits in UK pulse crops The 11th Annual International Rural Development Seminar: Legumes Sustainable Agriculture and Food Security. Boutflour Hall, Royal Agricultural University, Cirencester, 11 February 2015

DOMONEY, C. Presentation on PCGIN project to Lord de Mauley. Visit of Lord de Mauley, John Innes Centre, 6 March 2015

DOMONEY, C. CAP Implementation Seminar, CLA, Wingfield Barns, Wingfield, Suffolk, 12 March 2015

MARÍN-MANZANO M.C., ARQUES M.C., OLÍAS R., RUBIO, L.A., DOMONEY C. and CLEMENTE A. Fourth International Conference on Food Digestion, Naples, 17-19 March, 2015, p. 147

DOMONEY, C., CHINOY, C., BELL, A., RAYNER, T., ISAAC, P.G., SPANNER, R., MAGUIRE, R. and CLEMENTE, A. Plant Proteins for the Future, EUCARPIA International Symposium on Protein Crops, Pontevedra, Spain, 4-7 May 2015

CLEMENTE, A. and DOMONEY, C. Plant Proteins for the Future, EUCARPIA International Symposium on Protein Crops, Pontevedra, Spain, 4-7 May 2015

OTHER DISSEMINATION ACTIVITIES

O'SULLIVAN, D. Association of Science Teachers Conference: Legumes in the classroom, available at: http://www.ase.org.uk/documents/ac15-resource-using-peas-and-beans-to-teach-mendels-laws/ (2015)

DOMONEY, C., GRIFFITHS, S., OSTERGAARD, L., AMBROSE, M., WELLS, R., CLARKE, J. and JAMIESON, G. AgriTech/Defra GIN meeting & tour, JIC 2015

DOMONEY, C. BBSRC-industry meeting, Cereals Show, Lincolnshire, 2015

DOMONEY, C. Royal Norfolk Show, Farming Today & TV interviews; AgriTech-minister meeting; Mendel pea activity with SAW Trust, 2015

DOMONEY, C., GRIFFITHS, S., UAUY, C., MUMFORD, C., THOMAS, C. and SANDERS, D. Bawburgh Residents meeting, JIC, 2015

DOMONEY, C., SANDERS, D., GRIFFITHS, S., BEVAN, M. and SMITH, A.M.S. Lord de Mauley visit, JIC, 2015

DOMONEY, C., WILDE, G., MORRISON, D., SAUNDERS, N., SALT, L., NARBAD, A., HAZARD, B. and FROST, G. CRESTAR (Using crop genetics to understand the importance of dietary resistant starches for maintaining healthy glucose homeostasis) project meeting, JIC & IFR, 2015

(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.

See WP1 for dissemination activities. In additon, germplasm requests have been handled via the appropriate MTA route. In particular, the novel pea TI mutant line has been requested by several industries. (Where possible, BC lines are delivered, rather than exotic lines).

Future work

12. Please comment briefly on any new evidence 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	30/10/15
Position held	Head of Department, Metabolic Biology, John Innes Centre, Norwich Research Park		