

**Summary of PCGIN stakeholder's meeting
on 20th September 2005 at NIAB, Cambridge**

Attendees:

JIC	Noel Ellis Claire Domoney Mike Ambrose Catherine Chinoy David Holden Mary Wade
PGRO	Geoffrey Gent Anthony Biddle
NIAB	Jane Thomas Simon Kightley Haidee Philpott
SASA	Niall Green
CSL York	Adrian Charlton
Advanta Seeds	Keith Fox
Advanta Seeds	Simon Berry
Unilever	Frances Bligh
Cebeco-Seeds	Stephen Smith
Biogemma	Tina Barsby
IGER UK	Michael Abberton
ADAS	Richard Safford
University of York	Michael Schulze
University of Nottingham	Julian Wiseman
Pulse breeding consultant	Mervyn Pope
CCFRA	Richard Stanley
Pulse Trials Advisory Committee	Robert Pickard
BEPA	Andy Donald
Premium Crops	Edward Willmott
Birds Eye	Andrew Whiting
CPB Twyford	Liz Williams
Harper Adams University College	Robert B Wilkinson

Programme	Discussion leader
11:00 - 11:30 Chair: Geoffrey Gent (PGRO) Defra's aims with the crop genetic improvement networks and the PCGIN project: summary of the proposal and related projects.	Noel Ellis (JIC)
11:30 - 11:50 Objective 1: Communication and Delivery Events and progress to date: invited comments.	Claire Domoney (JIC)
11:50 - 12:10 Objective 2: Phenotyping Current activities & Comments from NIAB and PGRO (Geoffrey Gent PGRO, Jane Thomas NIAB) Invited comments	Mike Ambrose (JIC)
12:10 - 12:30 Objective 3: Performance Selection of lines Genotyping (Noel Ellis JIC) - Discussion of stakeholder involvement.	Jane Thomas (NIAB)
12:30 - 12:50 Objective 4: Reverse genetics Current report	Noel Ellis (JIC)

	Discussion of selection of mutants & lines	
12:50 - 14:00	Break for lunch and general discussions (Demonstration of plant materials , FTA [®] sampling and screening techniques) Chair: Jane Thomas (NIAB)	
14:00 - 14:10	Objective 5: Genetic mapping in crop legumes Update	Noel Ellis (JIC)
14:10 - 14:20	Objective 6: Genetics of Seed Quality Traits Outline of plans	Claire Domoney (JIC)
14:20 - 14:40	<i>Vicia faba</i> General discussion on the way forward	Geoffrey Gent (PGRO)
14:40 - 14:50	Satellite project	Adrian Charlton (CSL)
14:40 - 16:00	Round table discussion	
16:00 - 16:10	Concluding Comments	

All presentations are available separately on PCGIN web site (Meetings / Stakeholders' meeting 20th September 2005) as PDF files <http://www.pcg.in.org/meetings.htm>

Summary of meeting:

The objective of the meeting is threefold:

1. To inform participants of activities and progress and invite comment
2. To seek guidance on the selection of material for several analyses
3. To identify missing activities

Defra's aims with the crop genetic improvement networks and the PCGIN project

- **To support sustainable development of the arable sector,**
 - **By investing in public sector research**
 - **Enabling better collaboration between the strong UK research base and the plant breeding industry.**
- a) This is achieved by making available existing and generating new genomic tools and knowledge, in order to promote uptake of genomic technology
 - b) Resources: Creating stakeholders' fora through which all interested parties can provide input and obtain information on the activities of the Networks
 - c) Communication: The longer-term vision is that these Networks will help build critical mass in relevant applied strategic research in the UK so that the relevant teams become key to international 'public good' crop breeding research and can be sustained in the long term by a diverse funding base.
 - d) Organisation: The structure of the largest Networks supports three types of projects:
 - A large core project, that will generate, and make available in the public domain, technologies, genetic resources and expertise. This project has to have a management team comprising the research providers and funders with the responsibility for the science in the core project and management of the Network; and a stakeholders' forum, to promote debate, knowledge dissemination and feedback to the management team.
 - Specific, time-bound projects on traits, subjects and technologies identified as priorities, funded by Defra and other public research sponsors. These will generate more specific information and resources that will feed into the core project.
 - LINK projects led by the industry and that bring the research forward to exploitation.
 - e) Other 'GINs include those on wheat, oilseed rape and short rotation coppice; additionally, there is a Defra-funded crop plant transformation platform.

Comments:

TB raised the issue of the funding of the GLIP-derived technology transfer platform, GL-TTP. NE explained that the establishment of the platform was financed by the EU project, but the running costs of the platform were independent and the platform is intended to be self-financing. In part, these costs will be met by a subscription fee.

(see <http://www.eugrainlegumes.org/documents/TTPDescription.htm> and http://www.grainlegumes.com/default.asp?id_biblio=389)

Objective 1: Communication and Delivery. Events and progress to date: invited comments

- a) Establish and promote good communication between UK breeders in the pulse crops and the research base, both basic and applied, and ensure all have the opportunity to provide feedback throughout the project
- b) Establish a close liaison with SASA to ensure coordination and integration of our activities
- c) Establish a database of ongoing research in the area of pulse crops especially in the UK, but also at an international level
- d) Establish a web site, updated every 4 months and linked to the other Defra crop improvement networks
- e) Enable interaction days for management and stakeholders groups, providing opportunities to view and discuss genetic material
- f) Develop associated projects linking participants

Comments:

Progress under each heading was reported by CD. A question was raised by KF about legume research databases and the timescale for development within PCGIN. A lot of information on pulse crop research is available already but needs a structure i.e. database. Suggestions on structure and keywords etc. for database construction are needed from the stakeholders. There is therefore a requirement for a link on the website for messages, information and feedback.

The PCGIN web site has just been established (www.pcgin.org) and functionality will be added but this needs some input from an informatician. Some such costs can be paid by the PCGIN and will be developed when the data are available. In the meantime the PCGIN stakeholders should be aware of the GERMINATE database that accommodates some of the information relevant to PCGIN.

(See <http://germinate.scri.sari.ac.uk/germinate/>).

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Objective 2: Phenotyping

- a) Evaluating priority traits for different species by members of the PCGIN, taking account of the assessment of priorities already indicated by the breeders' survey
- b) Making informed choices on the species, germplasm, traits and specific biotic stresses to be studied, to include the use of exotic germplasm
- c) Developing a series of protocols for assessment of the traits required, with rapid and reproducible scoring techniques and reliable methodology for G x E interactions established at three sites (NIAB, JIC, PGRO)
- d) Correlating genetic marker data, where available, with phenotypic characters

MA presented a summary of the work already undertaken to meet this objective.

- i) Collect phenotypic data from pea material that has already been extensively genotyped
- ii) Extend the current data sets at NIAB, PGRO and SASA to exotic cultivated pea germplasm to complement the existing UK and Western European cultivar data (objective 3)

- iii) Develop common protocols for plot husbandry and data recording for priority traits

Forty-seven exotic cultivated (*P. sativum*) germplasm accessions had been selected on the basis of prior molecular and phenotypic information to provide a wide set of contrasting lines. This material was sown as microplots at JIC as a multiplication exercise and to enable PCGIN partners who will be involved with growing a smaller subset of this material in subsequent years to view the material. Seeds from six of the lines were sown also at NIAB and PGRO as a pre-trial.

Following a meeting at JIC on July 1st (www.pcgin.org/meetings), issues associated with plot husbandry had been discussed and common approaches agreed. A draft phenotypic score sheet to be used across the three sites was presented.

(see PCGIN website/Meetings/Stakeholders meeting 20th September 2005)

<http://www.pcgin.org/meetings.htm>

Comments:

KF raised the question as to whether or not there would be replication of plots at every location. MA replied that limited replication was planned. The extent of this depended very much on the seed returns from this year's multiplication work.

KF also asked if modern reference cultivars could be included to enable results to be set against known commercial material, since scoring 1 – 5 could be very subjective. It was agreed that inclusion of a commercial vining, and a field, pea variety would facilitate scoring comparisons.

A question was asked as to why such extreme exotic material was being included and how the 47 lines were chosen. The aim was to look outside the usual breeding pool in order to look for novel variation/ traits associated with canopy architecture and standing ability and to try to match genotypically diverse germplasm with phenotypic observations. Lots of genetic data are available for the chosen lines, which can be exploited in order to widen the phenotypic range of characteristics. The selection of the 47 lines was done on the basis of sampling across the major groupings within cultivated peas, using the results of molecular diversity studies carried out on extended sets of germplasm from the JIC Pisum collection. (see PCGIN website/Meetings/Stakeholders meeting 20th September 2005) <http://www.pcgin.org/meetings.htm>

A further question related to the scoring of agronomic traits only. The work within this objective is open to suggestions on desirable characteristics for end product and seed usability. This overlaps with Objective 6 and definition of desirable traits depends on feed-back from end-users.

Current activities & Comments from NIAB and PGRO

Comments from NIAB (Jane Thomas)

A set of six lines was provided from JIC for initial observation and recording, and to allow familiarisation with exotic germplasm. There was sufficient seed for 3 row plots, and although seed was sown late in April, all lines except one established and plots developed a canopy. The area was hand-weeded, although all the material is expected to be tolerant of current herbicides. One spray was applied to control weevils but damage to leaves was severe and, in one line with slow germination, all plants eventually died, probably due to weevil damage. Control of this pest will be critical for the main trial to begin in 2006. Various measurements of branching, haulm length, pod and seed number, and seed weight were taken. These will be compared with corresponding observations from JIC and PGRO. NIAB staff attended a summer meeting at JIC (<http://www.pcgin.org/meetings.htm>) to plan the design and protocols for the main trial with 20 lines in 2006.

Comments from PGRO (Anthony Biddle)

AB reported briefly on the growing of the same six exotic lines at PGRO. Similar germination and pest problems were experienced but these would be controlled in the future.

Objective 3: Performance

- a) Selection of maximally informative databases for UK pulse crop characters, site characteristics, history and yearly records
- b) Selecting a set of 50 cultivars that are differential for the traits identified in the assessment of breeders' priorities (yield, standing ability, disease resistance), based on maximally informative databases
- c) Genotyping selected cultivars with genetic markers (200 each)
- d) Selecting three cultivars that are maximally informative on the basis of phenotypic data and marker analysis for the generation of segregating populations
- e) Establishing recombinant inbred lines (RILs) to F₆ from crosses between the chosen lines (150 lines per population)

Comments:

Variety mean agronomic, disease and quality characters were pulled from the Grain Pea database, for the years 1988-2004 and for all trials that had acceptable yields. These data were analysed using BI-plot analysis to look for varieties that suggested they were outliers to the majority of varieties, for some characteristics. Firstly, the data were analysed as a complete dataset to gain some impression of how closely performing the varieties are to each other. The data were then split into three-year periods, with a year overlap, with the BI-plot analyses being repeated for each period. These smaller datasets allow outliers within them to be more easily seen. Character groupings, for the dataset, are also shown on the BI-plot print out with closely correlated characters near each other on the 'line'. (This 'line' is actually a circle opened out. Characters will be close to each other if they appear at the top and bottom of the line as well as next to each other.) A list of the outlying varieties, from the BI-plot analyses, has been compiled which suggests some varieties that might be of interest to study further.

A **proposed action** for the PCGIN is to circulate a list of cultivars from which the 50 to be genotyped will be selected and comments sought. It was suggested that a sub-committee is formed to consider this – please contact NE if you are interested in participating in this activity.

There was discussion among participants of the meeting on the characteristics needed in these cultivars and how best to interact in making the final choices. It was noted that this needs to be done soon, and should be in place by the time that the RA appointment at JIC is made.

AB commented that there were 50 lines available, using vining pea and combining pea data. It was noted that the phenotypic contrasts needed to be large. GG commented that there was a changing situation in agriculture and notably for the pea crops. With the selection of the 50 lines, it was important to anticipate phenotypic needs in 5 years' time.

AD commented that as farms got bigger, pea management was more difficult, and therefore one of the principal characters needed was ease of combining and standing ability. This point was well-noted and relates in part to the general issue of standing ability and plant architecture. It will be investigated whether or not the NIAB data set includes such information but, in the absence of this, comments on experience at the farm level would be welcome (to NE: noel.ellis@bbsrc.ac.uk).

It was pointed out by CD that the characters of greatest significance to PCGIN are based on a desk-top sustainability study at NIAB and the breeders' survey carried out for the PCGIN proposal; the results of both of these were in general agreement on priorities.

Further queries related to a need for a list of different end-users. In part, this issue will be dealt with by the database listing of who does what, that is in preparation.

Objective 4: Reverse genetics

TILLING population: Access through GL-TTP

Needs: Selection of candidate genes

- a) Access to ca. 5000 pea cDNA sequences (ESTs)
- b) Annotating sequences and identifying targets for TILLING, based on database mining and identity of orthologous genes involved in plant shoot architecture
- c) Designing primers based on candidate genes, derived from a) and b) and from databases developed within the EU project (Grain Legumes)
- d) TILLING for mutants in these genes, using the platform developed within the EU project (Grain Legumes) and assessing phenotypes of mutants

Comments:

NE asked participants for suggestions regarding structural genes where the mutant phenotype may be of interest to the PCGIN stakeholders. One response related to looking for altered starch variety mutants in peas. A set of starch mutants in pea has been isolated in previous work at JIC (and elsewhere) and these mutants are available through the JIC *Pisum* germplasm collection (<http://www.jic.bbsrc.ac.uk/germplas/pisum/index.htm>).

It was proposed (and agreed) that information could be assembled through a closed page on the website. The establishment of a LINK project in this area was proposed and it was agreed that, if the outline objectives could be established by the stakeholder group, this would add strength to such a proposal. This type of development is entirely what is anticipated as an outcome from the Crop Genetic Improvement Networks.

[During the lunch-break, there was a demonstration of plant materials and screening techniques. See *below for a report on this activity.]

Objective 5: Genetic mapping in crop legumes - Update

Objective 5a Provide novel germplasm for trait analysis (Fast Neutron mutant analysis and mapping)

- a) Selecting a set of 12 independent M3 individuals from a novel legume genetic resource [fast neutron (FN) deletion pea population]
- b) Performing cDNA-AFLP analysis of gene transcripts and determining the number of genes deleted per FN line.
- c) Sequencing the cDNA-AFLP fragments corresponding to the missing transcripts and identifying the corresponding genes
- d) Performing marker analysis of the deleted fragments by mapping identified genes in extant mapping RI populations

Objective 5b Integrated Genetic Maps

- a) Identifying the most effective strategy for relating the genetic maps of pea, field bean and lupin for UK use
- b) Establishing genetic mapping populations for bean and lupin within UK through coordination with the EU Grain Legumes project
- c) Designing a comprehensive set of gene-based markers (at least 100) to enable integration of gene maps across crop species
- d) Defining a set of molecular markers for priority traits that can be exploited by breeders

Comments:

The issue of how to proceed with the field bean component of the work was discussed. One likely strategy, but still to be agreed, was to initiate the development of a PCGIN mapping population based on selections from the small germplasm collection available at JIC.

For lupins, it was commented that all material grown in the UK was of one variety. This narrow genetic base posed a potential problem.

The issue of including clover within the remit of the PCGIN was raised. NE explained that this idea was central to the original PCGIN proposal, and that gathering all UK crop legume genetics under one umbrella remained an aspiration. Unfortunately, this structure was disallowed by Defra at the time of the PCGIN application.

It was commented that the rules for agriculture in the EU will likely change and that lupins will probably become a much more attractive crop in the future in addition to peas and beans. There is therefore a need for PCGIN to look at a more broadened range of crops. This point is well-made and, within the description of PCGIN, the issue is discussed. However, PCGIN has to set priorities governed largely by what can be done at the moment. It is recognised that positioning the group to be able to take advantage of advances, for example in lupin comparative genetics undertaken in the GLIP project is an enabling activity that should be undertaken.

With regard to emergent genetic and molecular maps, SB queried if it would be possible to have information available through the web-site. This will be considered under web-site development.

Objective 6: Genetics of Seed Quality Traits - Outline of plans

- 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
- e) *De novo* satellite projects with industry to exploit this variation

Comments:

The expansion of stakeholders to include end-users was discussed; invitations to the meeting had been issued through JIC, Advanta and PGRO. It was necessary, therefore, to combine the information on end-user participation, with respect to those who attended, those who could not attend but wished to be kept informed, and those who may have been omitted (inadvertently). The goal is to be as inclusive as possible in our discussions.

The queries that had been raised by end-users and industry in relation to quality were listed. Recent progress in genetic and biochemical methodologies was reviewed (and see Satellite project below). There was discussion on the different definitions of quality and how this is driven by economic forces. In the case of feed nutritional value, the animal sector is driven by growth performance, in contrast to quality for human consumption, which is driven by taste, smell etc. JW commented on some misconceptions on the requirements for animal nutrition. MP pointed out that the primary limitations for animal feed were different in UK compared with other EU countries, particularly France, where anti-nutritional protein content was considered to be of prime importance.

LW raised the issue of improving protein content overall in European pulses, particularly pea. CD pointed out this can be done genetically but only by a certain amount. New approaches should be explored to achieve greater increases. The point was made that the animal industry doesn't use peas because of variability, with environmental factors having a great effect. Beans were mentioned briefly in relation to their tannin content; anti-nutrients in beans are being investigated within research programmes in France.

There was a brief discussion on the possible role of pulses in biofuel production. SK pointed out the limited land-use within the UK available for this. However, queries had been raised earlier by Wessex Grain Ltd in relation to the availability of high-starch crops, requiring no nitrogen fertiliser, which could be used for bioethanol. RS (ADAS) expressed interest in any feasibility work looking at the development or use of high-starch peas for bioethanol production. This would be a good candidate for a proposed 'spin-off' LINK programme.

Prioritisation of the various quality issues is needed, probably through a smaller sub-group linking with stakeholders. This could be managed through a closed page of the web-site, as discussed above (Objective 4).

***Vicia faba* - General discussion on the way forward**

PCGIN role: Mapping Population – choice of parents
Markers – available through Univ. Dundee, GLIP & GL-TTP and EuFaba

Comments:

GG led this discussion and reminded participants of the agricultural significance of the field bean crop and the way that it had expanded, partly at the expense of peas. He reviewed the factors that made beans attractive to growers and the factors that could depress yields.

During discussion the following points were agreed;

- i) that winter and spring beans had to be considered as separate crops
- ii) that the variety screen established by MA was useful
- iii) that some traits that had been introduced but not found to be commercially useful in the 70's/80's could be re-assessed. These included compressed internodes and node specific vascular systems.
- iv) other traits would not be pursued, including tannin free and determinate beans.

Vicine and convicine content of beans were discussed briefly in relation to research at INRA, France (see Objective 6).

MP was of the view that beans now merited the same level of attention as peas and there was general support for the point that other pulse crops should be considered given the changing requirements of post-crop specific support agriculture. EW gave a possible scenario for the future, where in the absence of sugar beet, lime usage for arable land would be limited, so creating ideal conditions for white lupins.

GG agreed to liaise with JIC personnel and expressed his gratitude for the level of support and co operation given in the past.

Satellite project

AC (CSL) gave a brief overview of the metabolite profiling that has been undertaken in conjunction with JIC to define metabolites that change in response to a set of environmental conditions. This work has examined leaves and seeds from pea plants subjected to well-watered or drought conditions. These data relate clearly to quality issues discussed under Objective 6 and there was some discussion as to the identity of particular metabolites that change, how many of these had been identified already, and the development of a library of such compounds.

*** Lunch-time practical activity**

During the lunch-break, there was a demonstration of plant materials and screening techniques. Some pea variants and different bean varieties were on display. The pea variants included back-cross lines, segregating for quantity of a seed protein with allergenic properties, and lines derived from a fast-neutron mutagenised population exhibiting novel phenotypic traits including bulbous stem base, tendril-less, lathyroides and creeping stems.

There was an opportunity for attendees to sample leaves from plants and prepare leaf samples for marker analysis, using a simplified procedure that is applicable to field use and facile to transport/store. CC explained the procedures used and provided protocols and notes.