Pulse Crop Genetic Improvement Network:

Abstract:

Pulse crops and Defra policy objectives:

Crop diversification and lowering inputs are major drivers for sustainable agricultural policy. Pulse crops can contribute positively to these two policy goals because, as a consequence of biological nitrogen fixation, they require minimal inputs while acting as a very effective break crop in a cereal dominated rotation. Pulse crops are also an efficient source of plant-derived protein for food and animal feed, and thus meet a need that has grown since the BSE crisis. However, pulse crops are currently under-represented in the UK, and throughout Europe, despite their being essential for sustainability in agriculture.

A major cause of this limited contribution is farmers' perception that these crops have erratic performance and that their end product value is low. Yield potential of pulse crops is high but average yields are about half of the best achieved, and the best UK yields fall short of current yield potential by about 25%. There are many causes for this, but efforts to improve yield consistency through genetics and breeding have the potential to achieve an increase in pulse production in the UK.

The pulse crops, especially pea, can benefit from their association with the vegetable market where vining peas are a high value product. As the same species is involved in both markets (that are approximately of equivalent monetary value) the development of basic genetic tools can be a common resource, and the costs of their deployment can be shared.

Objectives of the Network:

The Defra Pulse Crop Genetic Improvement Network (PCGIN) will establish a platform that serves the process of legume crop improvement in the UK.

It will:

a. formally establish the route by which scientific resources, results and knowledge will be delivered to breeders, producers and end users, providing a link between these groups and the research base to achieve added value for pulse crops,
b. provide resources, expertise and understanding that will be drawn upon by both public and commercial sectors in breeding, analysis, and in the definition and improvement of product quality relating to both commercial and public goods,
c. promote and execute the translation of genomic research tools to crop improvement, consistent with both the needs of UK industry, and Defra objectives relating to sustainable agriculture,
d. provide a direct link with, and involvement in, European pulse crop research programmes.

The PCGIN will be managed by JIC, PGRO, NIAB and Defra, with *ex officio* input provided by the commercial sector, initially Unilever plc and Advanta Seeds UK. Core scientific research underpinning pulse crop genetics will be performed jointly by the first three organisations in projects that combine phenotypic and performance character assessment with genetic tool development. The Management Group will oversee an integrated set of related projects that exploit the activity of the core programme.

Purpose:

Why should Defra support a pulse crop genetic improvement network?

Pulse crops benefit UK agriculture

Pulse crops are an efficient source of plant-derived protein that, as a consequence of biological nitrogen fixation, require minimal inputs and act as a very effective break crop. Recent PGRO data on the relative importance of individual legume species to UK agriculture shows two major and two minor species: pea (50%), *Vicia* bean (40%), lupin (5%) and *Phaseolus* bean (5%). The economics of the UK pea crop are elaborated upon below, since the benefits of pea relate to the value of the vining pea for UK industry and human consumption as well as the separate value of the dried pea crop. Clearly the *Vicia* bean crop is grown currently at a lower acreage than the total pea crop but grower interest has shifted in recent years towards bean and these figures describe the current status of the UK pulse crops. However, legume crops are currently under-represented in the UK and throughout Europe and yet are essential to sustainability in agriculture. Thus the monetary value of pulse crops is significantly underestimated by consideration of market value figures alone: see Section 9 for discussion on environmental issues.

Pulse crops make efficient use of resources. Approximately a third to a half of the energy inputs to arable agriculture are associated with the production and use of nitrogen (N) fertilizers. Pulse crops do not make this energy demand and fix nitrogen over a sustained period. Pulse crops improve the N status of soil without many of the run-off problems associated with fertilisers, and biologically fixed nitrogen has a different pattern of availability in the soil from exogenously applied chemical fertiliser. Water-use efficiency of pulse crops is high, making minimal demands on irrigation. Thus pulse crops are an energy efficient break crop with a low environmental impact. Increasing the incorporation of legume crops in UK agriculture will improve energy efficiency and minimise inputs, both in terms of exogenous fertiliser and as a component of disease control through the break crop effect.

Pulse crops partly meet UK demand. The UK vining pea market is the largest in Europe and this market can be met by UK production provided that the timing of harvest is adequately controlled. Improvement in the control of harvest timing would markedly improve the efficiency of harvest of this crop. For the combining pea and bean crops, demand is higher than the level of production with most of the additional demand being replaced by imported soybean meal. This demand relates mainly to the use of pulse crops for animal feed manufacture but also to more speciality markets where dried pulses are incorporated into plant protein products for human consumption. Even a substantial increase in UK grown pulses to meet these demands would have a minor impact on soybean import.

The problems associated with pulse crops for UK agriculture

In order for PCGIN to address priority problems associated with pulse crops in UK, Dr K. Fox (Advanta Seeds UK, Lincoln and BSPB representative for pulse crops) has asked the UK breeders to assess areas in which research needs are greatest and to assess the common limitations to the major legume species grown in UK. This analysis addressed the requirements of both pea and bean as major crops, and lupin as a minor legume crop. The results of this survey are discussed in further detail and presented in Section 9 of this proposal (Constraints). These show that the major priorities and, by inference, obstacles to increased pulse production in the UK, are factors controlling yield and yield stability. These represented the common priorities for all the legume crops and, being complex traits, are linked, from a research viewpoint, to factors controlling architecture of the plant, disease susceptibility and abiotic stress resistance. Additional priorities were associated with individual crops, with diverse species-specific disease problems. A major priority for beans was identified as drought tolerance; the relative drought sensitivity of varieties of beans currently grown appears to represent an obstacle to increased use. In addition to breeders' priorities, further obstacles to UK pulse crop use are perceived by end-users. Erratic supply of legume seeds results in imports (from France and Belgium) that could/should be met by UK. Poor regulation of product quality for animal feed means that improved varieties are not exploited as expected. Thus research is required that addresses the issues underlying these problems and limitations, as well as providing the resources needed to maintain and increase agricultural production and value of legumes while maintaining the advantages of minimal input requirements.

How to address the problems associated with pulse crops for UK agriculture
The PCGIN is proposed as a Defra-funded platform to enable coordination of the aims of the pulse crop breeders and end-users with those of the research community, and thus to establish a pipeline for delivering genetic knowledge and improvement to UK legume crops. Defra support is vital to establish this critical link and to support the underpinning pre-competitive research that is necessary to support legume crop improvement to meet specific UK needs. A formal structure to link the research base with breeders and end-users, to focus research in areas of highest importance and to ensure take-up of research outputs with maximal benefit has hitherto not existed within UK. A parallel structure is in place in France (and Germany) and is deemed to operate with a high level of success in providing research to back integrated and targeted priorities.

Proposed PCGIN Structure
The Network is proposed as an open structure, with several layers of activity. The network will have a core research group composed of JIC, NIAB, PGRO and Defra. These organisations will establish jointly a Management group (with commercial groups such as Unilever and Advanta having *ex officio* roles) to target and guide research on pulse crop genetics to underpin legume crop improvement to meet UK demands. The research will be undertaken jointly by the John Innes Centre, NIAB and PGRO. The core group will establish an integrated set of related projects to exploit the activities of the core programme. PCGIN will assemble the relevant scientific and end user communities in the UK as a 'stakeholders' forum as a means for the interaction, discussion and dissemination of network activities. The 'stakeholders' forum will ensure a pipeline for the delivery of research goods and knowledge.

The PCGIN proposes to address the problems and priorities for pulse crops, identified in consultation with breeders and end-users, by establishing an initial set of core scientific activities. These activities are described here with some detail provided for pea, since this is where genetic expertise currently lies within the UK. However, it is proposed to include *Vicia* by defining priorities, scope and resources as an early deliverable. Lupin will be included in objective 4, and will be coordinated with Defra sponsored work at IGER (LK954)

How does the consortium focus on research that will result in improvement of pulse crops through improved crop breeding?

The proposed network is intended to foster a research alliance in pulse crop genetics. To this end its role is to engender cooperation in the development and deployment of genetic resources. Previously the basic genetics of the pea crop has been the responsibility of JIC, while pulse breeding more generally has been the responsibility of commercial organisations, represented at the establishment of the PCGIN by Advanta and Unilever. The independent monitoring and assessment of these crops has been undertaken by NIAB, and SASA in Scotland, while PGRO was established to improve the growing, harvesting and use of the dried pea crop, but is now concerned with Research and Development to improve the reliability, agronomy and value of pulse crops generally, especially peas, field beans and lupins.
These differing roles can be made to integrate more effectively if there is a set of common goals and resources shared by these distinct bodies. The PCGIN will generate these through its shared responsibility for pulse crop genetics. Phenotypic characterisation is key to these activities and the establishment of a common approach that relates field performance or processing characteristics to single plant characters that are amenable to genetic analysis is essential to the successful exploitation of genetics in breeding. The PCGIN will draw on JIC’s experience in pea genetics and its engagement with field bean and lupin genetics outside the UK. The trait targets for genetic analysis within the PCGIN will be determined by a combination of tractability and relevance, the latter being strongly informed by the experience of NIAB and PGRO, with additional input being sought from SASA. The PCGIN’s understanding of the relevance of these traits will also be informed by the stakeholder group. The output of the PCGIN will not be cultivars, but genetic resources and tools: markers, plant genotypes and the understanding of the genetic control of relevant traits that can be exploited in breeding strategies. Again these need to be in a format that is useable by the stakeholder community and it is the function of the PCGIN management group to ensure that this is achieved. For the pea crop many of these resources can easily be envisaged, while for field bean ad lupin this is less clear because of the comparative lack of experience in the genetics of species in the UK. The PCGIN will therefore draw on comparative genetics as much as is possible and will produce a stakeholder driven plan for field bean genetics as an output in the first year.

The core scientific projects will be based on:

1. Phenotyping
2. Performance
3. Reverse Genetics
4. Genetic Mapping
5. Seed Quality

1. Phenotyping* to establish a common approach to the evaluation of germplasm, with particular attention to diverse germplasm selected on the basis of extensive genotype data. This material is often 'exotic' germplasm and trialling procedures may need to be adapted according to the source of the material. This objective will address the current interest of breeders in exotic germplasm as a source of novel genes.

2. Performance* data for modern cultivars are available but this material has never been extensively genotyped. The project will undertake genotyping of this material and establish Recombinant Inbred Populations from informative crosses between cultivars of contrasting performance to establish the genetic basis for priority traits.

3. Reverse Genetics tools will be developed and exploited to provide access to genes regulating traits of interest to UK pulse crops. This approach will identify and confirm the role played by candidate genes in traits that are simply inherited, for example genes controlling some aspects of plant architecture or drought tolerance. Genes proposed to exert major effects on more complex traits, e.g. quantitative trait loci (QTL) controlling standing ability and yield parameters may also be investigated in this way.

4. Genetic mapping will integrate genetic maps developed within the Network with emerging sequence data from legume genome sequencing projects, and will test the feasibility of generating an ordered set of deletion mutants for one UK pulse crop to enable gene identification associated with QTL. The mapping activities will be closely integrated with European projects to maximise the benefit to UK priorities; for example, populations already established within a European *Vicia* project (EU-FABA) will be exploited to identify genes for UK priority traits and synteny among maps of closely-related legumes will be exploited, thus providing Defra and UK agriculture alike with added-value from Defra investment.

5. Quality traits in the dry seed used for animal feed will be the initial focus of investigation, with specific reference to quality issues and associated perceptions that limit the current exploitation of the UK pulse crops in animal feed. Defining these parameters will necessarily involve consultation with feed manufacturers and will set quality standards that provide assurance and the means to improve end use quality in breeding programmes. This consultation will include additional, and potential, end-users of legume seed products (e.g. manufacturers of bio-fuel and human food).

***Note** that 1 and 2 provide the direct means to link genetic results obtained with laboratory lines to genotypes selected on the basis of phenotypic 'superiority' in the field, thus enabling the identification of genes and markers associated with agronomic traits not hitherto investigated at a molecular level.

Scientific Context

Background

Pulse crops are minor crops in a UK context but have increased somewhat in significance since the mid 1990s from approx. 170,000 to 250,000 ha. As outlined in Section 8 above (Purpose), pulse crops could be utilised to a greater extent for economic as well as environmental and societal benefits. An increase in the utilisation of grain legumes within UK arable cropping systems would provide for greater self-sufficiency in protein for food and feed, increased symbiotic N_2 fixation with benefits for succeeding crops of N supply, as well as the beneifts derived from the break-crop effect. Grain legume cultivation is associated with lower inputs of fertiliser, pesticides and fossil energy in arable cropping systems. Consequently the sustainability of farming systems is increased and potential health risks associated with pesticide use and possible nitrate pollution of drinking water are reduced. Furthermore, farmers can potentially save expenditure on N-fertilizers and pesticides and there is a saving of non-renewable natural resources. New strategies for increased cultivation require improved cultivars, coupled with both environmental and economic analysis, to ensure that measurable benefits are achieved for farmers and the environment. The 'hidden' costs of food (and feed) production within the UK include costs associated with the removal of chemicals and pesticides from water, estimated to be currently £69M and £120M per annum, respectively. These costs are borne by the public and represent major environmental and public savings that could be made in more sustainable agricultural systems. Potentially, then, there is a great deal to be gained by investment in, and promotion of, pulse crops.

The status of the UK pulse crop

Pulse crops in the UK represent about 5% of the arable area, representing about 200,000 ha in England from 1997 to 2000; an increase to 270,000 ha in 2001 coincided with a downturn in the area under cereal cultivation. Increase in the area of pulses as break crops is not in direct competition with oilseed rape, which showed a larger increase in the same period. Approximately half of this area is pea (*Pisum sativum*) and slightly less is field bean (*Vicia faba*). The total value of the crop is about £65M (assuming a price of £80/t and a yield of 4t/ha). A pulse / wheat and a rape / wheat rotation should have similar overall returns to farmers, given the differences in input costs, area payments and final value. The farm gate value of the vining pea crop in the UK is about £37M. Thus the pulse crop in the UK has a value of approximately £100M p.a. This value is limited by the area under cultivation. For the vining pea crop this is determined by accessibility to processing plants, and for both the vining and combining crops the rotation period is limited largely by susceptibility to root diseases, and yield instability. In part, the small scale of pulse crops is probably due to preferred sowing dates, and to the historical legacy of support. The less favoured status of pulse crops is, nevertheless, puzzling. The input costs are lower than for oilseed rape for example and the area payment is also slightly higher. (The lower input costs derive largely from the nitrogen fixation of the pulse crop, and this provides environmental as well as economic benefit.) As far as the combining pea is concerned, the answer to this question seems to be that farmers do not have confidence in the relatively modest yield of 4t/ha; in France, yields are typically about 20% higher, and more so in the North. The reasons for the higher yields in northern France have not been investigated systematically but probably derive from a combination of factors. Notably, the area under pulse crop cultivation is much higher in France and the crop benefits from the associated economy of scale in relation to crop management. Lodging and flowering time remain important components of plant architecture that continue to constrain the UK pea crop. Yield potential is not the major factor limiting the exploitation of this crop; rather farmers' confidence is undermined by the potential for pre-harvest loss, typically from bird damage or lodging and disease. The situation with beans is quite different, in that fewer pre-harvest problems exist; lodging (standing ability) is not a major problem here but sensitivity to drought is much greater than for pea, with the latter possessing a good degree of tolerance. Where pulse crops are more frequent components of rotations, disease control problems can inhibit their more intensive use.

There are two potential solutions to the problem of scale of pulse crop use: increasing the value of the crop, to offset the potential financial loss, or attempting to ameliorate the problem of scale. Value is not a consequence of yield; rather it is a consequence of use and quality. For a given use and quality, increasing the total crop yield may not increase the return to the farmer if the consequence is to depress price. Quality issues determine the target market and thus the price that can be obtained for the crop (e.g. feed peas fetch a lower price than peas for food). However, competitivity of the crop in the market is affected by variation in yield; at a given market price, if the crop has a low yield the return is also low. Addressing both quality/use and yield/scale issues requires considerable research investment that is hard to justify for the breeder, given the value of the seed. A substantial benefit in the pulse/wheat rotation comes from the added value to the 'first' wheat crop, where a preceding legume crop increases the wheat yield by about 20% with no associated cost of N fertilizer. It is difficult for these economic and environmental benefits to drive the activities necessary for the promotion of lower input, and more sustainable rotations. The value of such a development is partly economic but also importantly environmental, through lower input farming.

Constraints

The PCGIN proposes to link the research base with the stakeholder needs for pulse crop improvement. In order to define this link for preparing the current proposal, a preliminary assessment of UK breeders' priorities, given in the Table below, was undertaken by Dr K. Fox, Advanta Seeds U.K. (28 breeders/agents were approached, of which a third (9) responded). This identified the principal targets for pulse crop improvement as: yield, yield stability, standing ability (as related to plant and crop architecture), disease (in particular Downy and Powdery Mildew, Foot and Root rots) and pest resistance (e.g. to aphids, considering their role as virus vectors). These constraints are consistently important for vining and combining peas, field beans and lupin. Clearly these targets are inter-related and contribute to minimising yield loss from yield potential. Standing ability, yield stability, nutrient use efficiency and plant architecture are all inter-connected and represent targets for which progress may be achieved, using the approaches described here.

Novel functionality of the seed was identified as a low priority but this is likely to be because there is no obvious added value return to breeders for such developments/improvements. Similarly new markets were given a 'medium' level of importance. These are likely to be connected to the undefined functionality and market; if these were defined, or an opportunity identified, the assessment of importance may change. This project will establish a 'stakeholders' forum to provide a direct connection and promote better communication between breeders, producers and end users, providing a route to achieve added-value for pulse crops. The PCGIN will thus provide the genetic means and resources to achieve this. While pulse crops can contribute positively to low input agriculture, the return here is in 'public goods' rather than in return to the farmer. There is clearly a need to gear added value to the farmer to improved public goods. Specifically, pulse crops act as a break crop in cereal rotations to minimise input costs while maintaining a high yield of the main cereal crop. Many different break crops are in use and, in a rotation, the production costs and financial return to the farmer need to be considered over the whole cycle. The advantage to a 'first' wheat crop following oilseed rape or pulse crop is similar. For pea and oilseed rape the gross margins are similar, but for a pulse crop the input costs are lower (as is the return). Thus for the same economic gain there is little difference, and farmers tend to choose the more stable return associated with rape rather than growing the lower input pulse crop where the return is less assured because of yield variability. Thus improving yield stability in the pulse crops will increase the likelihood that a farmer will grow a legume crop. However there is little financial incentive to the farmer but rather the total energy input of the cycle is lowered as is the total chemical input and N waste management. Thus the benefits are public goods which, although generated by the farmer making such a choice, are not of direct financial benefit to the farmer. However, these choices lead to an important pay-off when defined in terms of environmental benefit and sustainability.

Character	**Vining Peas**	**Combining Peas**	**Field beans**	**Lupins**
Yield	H	H	H	H
Standing ability	H	H	H	H
Eating quality of vining peas (vs. maturity and post harvest changes	H			
Plant architecture improvements (for weed control and harvesting benefits)	M	M	M (Spring) H (Winter)	M
Disease and pest resistance	H	H	H	H
Virus	M	M	L	
Nematode	L	L	H	
Foot and Root rots	H	H	L	
Downy Mildew	H	H	H	
Leaf and Pod Spot	M	M	M	
Drought tolerance	L	L	H (Spring) L (Winter)	L
Nutrient efficiency	L	L	L	L
Below ground - roots/nodulation	L	L	L	M
Use of legumes in sustainable rotations	M	M	M	M
Biodiversity - within and between crops	M	M	M	M
Winter crops	L	L	L	L
Spring crops	M	M	M	M
New markets for pulses	M	M	L	H
Improvement of minor pulses for marketability	L	M	M	H
Maintaining up-to-date view of UK pulse markets and opportunities	M	M	M	H
Novel functionalities	L	L	L	L

Importance:	H - High M - Medium L - Low

Related crops
Pulse crops and vegetable legumes are often the same species, as in the vining and combining pea crops. Similarly, broad beans are the vegetable equivalent of field beans. The vining pea crop has a high market value compared with the combining crop and, despite its much restricted growing area, it has a comparable total value to the combining pea crop. As the main route to crop improvement is through the application of genetics, the infrastructural investment in each single species that is grown both for arable and vegetable farming needs to be unified for both economy and efficiency. The PCGIN needs to address therefore both the pulse and vegetable crops.

Clearly there is more to be gained (per unit seed) by investment in vining pea breeding than in combining pea breeding, even though the economic and environmental impact in the combining pea market could be greater. The PCGIN requires input from the industrial sector involved in breeding both vegetable and combining crop types, and representation of the public sector interest in the promotion of a lower input agriculture that market forces alone will not drive.

Pea and field bean (*Vicia faba*) are the major legume crops in the UK, but pea genetics is currently more advanced[1 -5]. There has been a long standing interest in the potential for lupin as a UK grain legume crop (*Lupinus albus* and *L. angustifolius*), but this has not yet come to fruition. The initial focus of the PCGIN must therefore lie with pea and bean. In both cases, the management group will define and streamline priorities as the PCGIN develops. As a matter of urgency, the management group will develop a specific strategy for *Vicia faba*, for which UK genetic and infrastructural resources are minimal in comparison to pea; groundwork for this strategy is already in place through links within the EU (see International Dimension below). The PCGIN will build strong links to lupin work (at Reading and through a proposed EU training network project, coordinated by the Institute of Genetics, Poznan, where lupin is the main focus and to which JIC will provide materials). The PCGIN will monitor and review the need to include lupin species within its remit and will be guided by PGRO on its continuing or increasing relevance to UK agriculture. Similarly, *Phaseolus* is not planned to be within the scope of the Network, but this will also be reviewed. Forage legume crops will be discussed later.

Adding value
The vining pea crop *per se* could be regarded as 'adding value' to the pea crop. There is added value in that the typical price for vining peas is about £240 per tonne. Currently the value of combining peas is rather volatile but the best marrowfat samples are trading at £200 per tonne. In contrast feed values of peas are about £85 per tonne and field beans about £75 per tonne. Extracting this added value from a vining pea crop requires considerable investment, and for this reason the vining pea crop is largely restricted to areas close to freezing plants. Increasing the financial value of the crop is an objective in both sectors; a premium is paid for quality in the frozen vegetable market, and in some EU countries the quality of the dry seed crop is linked to the farmer's return[see 3]. In both cases seed quality controls the value. The dried pea crop is used mainly for animal feed, but as a food crop it has a higher value. The project will specifically address the issue of digestibility. For example, in the UK the current maximum inclusion rate for pea in piglet feed is 7.5%. This limit is not set in other European countries, and thus in the UK the field pea crop is limited

and disadvantaged in a way that is not the case elsewhere. It is necessary to define the quality attributes that determine this limit to inclusion rates so that the quality can be assured and so generate a larger potential market. Similar arguments apply to broiler and other animal feeds.

An International Dimension
The PCGIN is targeted at UK needs in relation to legumes, excluding forage species, in agriculture. However, the UK needs are not in isolation and are closely related to the needs of other European countries, as well as other regions with similar climatic conditions. There are several ways in which the PCGIN relates to activities external to the UK.

The lead institution (JIC) is the coordinator of an EU integrated project (Grain Legumes), involving 60 laboratories concerned with the development of tools for the improvement of grain legumes. This project has been approved and is in the final stages of contract negotiation. This connection between the PCGIN and Grain Legumes will provide PCGIN with direct access to information on a range of species, most significantly *Vicia faba* where, as stated above, infrastructure for genetical studies is lacking within UK. It is for reasons of efficiency that the development of a strategy for *Vicia* must take account of activities at an international level, principally to avoid replicating preliminary work that is relevant to PCGIN but that has been carried out elsewhere previously. The definition of the optimal approach for the PCGIN to the development and exploitation of *Vicia* resources for UK agriculture should be negotiated in the light of international activities and this should be defined as a matter of priority by the management group. For example, if drought sensitivity is a priority trait to be studied in this species (as indicated by breeders' survey), then existing genetic crosses may be appropriate and could be exploited collaboratively for phenotypic or genotypic screening. Specifically, a work package on abiotic stress within the EU programme may provide markers and resources that are applicable to *Vicia* and in this way provide added value to PCGIN. It is anticipated that the PCGIN and the EU Grain Legumes project will interact at a formal level for the exchange of information. The precise mechanism for this must await the establishment of both structures, but will be guaranteed by the common leading institution. Contributions to the EU project from JIC will include some specific activities within the PCGIN. The proposed studies on seed quality attributes will complement EU-funded work in relation to seed composition and will profit from access to metabolomic facilities that will be exploited for seed profiling within the EU programme. As JIC has a 'full cost' model with the EU, its proposed PCGIN activities will be combined as a contribution to the EU project. The PCGIN will similarly look to the comparative genetics component of the EU project in order to identify suitable genetic markers for field bean, and if appropriate lupin, and as a potential source of mapping populations to facilitate the development of markers and tools relevant to UK agriculture. Since the purpose of Defra’s Networks is to bridge the gap between genomics and breeding by providing tools for genetic analysis and breeding, including markers, well-characterised germplasm and knowledge, it is clear that participation within the larger EU framework will greatly facilitate these objectives.

Wider links, for example in the US, Australia and throughout the CGAIR system, will also be facilitated by individual contacts, such as the role of Mike Ambrose (JIC) in the European Cooperative Programme for Crop Genetic Resources (ECP/GR) as chair of the Grain Legume working group. Joint funding will be sought from other sources to further the joint interests of these groups and the PCGIN at an international level. The general objective of the PCGIN is to provide access to genetic variation for crop improvement through the matching of genotypic and phenotypic data and by exploiting comparative genetic map data. These objectives are closely allied to a 'Challenge Programme' being developed in association with the CGAIR that will relate mainly to CG-mandate crops. These do not include pea, faba bean and lupin that are the targets of the PCGIN; however, our approaches and methods will be similar and therefore links with the 'Challenge Programme' will be sought by the management team.

Conclusions

There is a clear need for pre-competitive research underpinning crop improvement in peas and beans that takes account of both main end uses and so underpins the development of a lower input, higher value arable and vegetable sector. This research needs to address the problems identified by breeders and end-users, and to identify the potential routes to add value to the crop while reducing risks of crop loss and so improve the use of legumes in sustainable crop rotations.

Scientific Objectives

Objective 1: Communication and Delivery

To establish and promote effective communication between the major players responsible for the genetic improvement of pulse crops. This first objective is not a scientific activity *per se* but it is essential to the relevance, application and delivery of the scientific activities of the PCGIN. Its activities are to

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

Objective 2: Phenotyping

Phenotypic characterisation of novel legume germplasm

This will involve

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

Objective 3: Performance

Extant varietal performance data will be associated with genotype data

This will be based on

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 F_6 from crosses between the chosen lines (150 lines per population).

Objective 4: Reverse genetics

TILLING *for genes that regulate the development of the aerial part of the legume plant*

This will involve

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.

Objective 5: Genetic mapping in crop legumes

5a Provide novel germplasm for trait analysis (Fast Neutron mutant analysis and mapping)
5b Integrated Genetic Maps

Objective 5a will be achieved by

a) selecting a set of 12 independent M_3 individuals from a novel legume genetic resource [fast neutron (FN) deletion pea population]
b) performing cDNA-AFLP analysis of gene transcripts from total plants of these individuals 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 will involve

e) identifying the most effective strategy for relating the genetic maps of pea, field bean and lupin for UK use
f) establishing genetic mapping populations for bean and lupin within UK through coordination with the EU Grain Legumes project
g) designing a comprehensive set of gene-based markers (at least 100) to enable integration of gene maps across crop species
h) defining a set of molecular markers for priority traits that can be exploited by breeders

Objective 6: Genetics of Seed Quality Traits

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

Interdependence of objectives:

All objectives are reliant on the success of objective 1.
Objectives 2 and 3 are related, but not inter-dependent. The varietal performance data utilised in objective 3 will inform the selection of some phenotypic characterisation in objective 2.
Objective 6 is dependent to some extent on objective 3 and to a lesser degree objective 2.
Otherwise the objectives are independent.

Factors that may cause delay:

None of the objectives is dependent on the development of novel methods and so all are considered to be robust.
Database incompatibility may be encountered within objective 3 but this is regarded as being a manageable issue.
Objective 4 depends on access to a TILLING platform within an EU FP6 project (Grain Legumes) that is currently funded. The TILLING platform will be available to PCGIN, although the EU project may set priorities that slow access by PCGIN but this seems unlikely provided funds for the TILLING screen are made available.
Objective 5 requires the use of established methods that are technically demanding and require the skills of an experienced person; this is a manageable issue.

Approaches and Research Plan

General comments on approaches in relation to PCGIN structure

A core programme in three parts is proposed:
1) A small network comprising the Management group.
2) Infrastructural scientific activity of the Network.
3) A collaborative Network.

From this core programme, additional projects will be developed. These will include LINK projects and bilateral collaborations, including CASE studentships. An initial measure of the success of PCGIN is that, during the preparation of this proposal, a BBSRC-funded CASE studentship has been awarded jointly to JIC and Unilever (start date: January 2005). In addition, through the role of JIC, the PCGIN will have direct access to the International Medicago Genome Sequencing project and an EU Framework 6 Integrated Project, Grain Legumes, that is now underway. The outcomes of these three activities will be presented to the Stakeholder Forum which will discuss, review and inform the direction of the programme

Project Management.
The success of the PCGIN will depend on the quality of the integration of its activities with the needs of the commercial sector and related public bodies, the impact of the Network on the activities of these groups, together with establishment of the pipeline for delivery of research to stakeholders. Our first objective must therefore be to establish good communication between UK breeders in the pulse crops with the research base, both basic and applied, and ensure they have the opportunity to provide feedback throughout the project; this is already underway with the involvement of Advanta Seeds in the preliminary survey reported in this proposal. A small management team formed by the contractors (JIC, PGRO and NIAB) and funder (Defra) will be responsible for running the core project, identifying priorities and setting the broad objectives for the Network, overseeing the relationship between the core PCGIN and associated projects, establishing communication with relevant parties and organising the 'Stakeholders' forum. The breeders and representatives from other relevant institutions will have *ex officio* representation at the meetings.

The extended group of the core PCGIN and associated projects will require a small budget to finance review and exchange meetings, as well as a degree of administrative support. The newly developed and associated projects will be LINK, CASE, BBSRC, EU-funded and Defra-funded satellite projects, either by commission or open competition; specific industrially-funded projects will also be sought.

We envisage that a wider group of interested parties will contribute to the direction of the core project. Their association is required to ensure the relevance of the core activities and the role of these bodies within the PCGIN will be subject to the discretion of the Management Group. We will establish a ‘Stakeholders' forum to include these bodies so that their input can be made to Project Management Group. This forum will include the breeders, SASA, and other bodies considered relevant by the Management Group. The 'Stakeholders’ forum will be open to relevant new members at any time.

Core activities of the Network
The core project will provide infrastructure and a knowledge base for legume crop improvement. These basic resources will enable the development of genetic and breeding approaches to address the target traits identified in the assessment of UK breeders' priorities (see above). In general, crop improvement relies on the effective exploitation of allelic variation and so the core activities must seek to develop analytical tools for the identification, accession and deployment of useful allelic variation in legume crops. The focus of core activities will be on pea and *Vicia* crops and will exploit emergent resources, developed elswhere within the EU Grain Legumes project, as applicable to both these and to lupin. The resources that will become available in this way are *Vicia* populations and the pea TILLING platform that will greatly accelerate mapping of UK priority traits determined by components of yield and plant architecture.

Network 'Membership'
This network must respond to changing needs from the market, producers and the research community, while fitting the priorities of Defra in relation to translating genomic tools to crop improvement for economic and environmental gain in a UK context. Thus 'membership' of the network will be open, flexible and capable of change; these changes will be reflected initially in the 'Stakeholders' forum but will also generate additional 'associated projects' that extend the scope of the network. A major mechanism by which this can be facilitated will be the development of associated projects and LINK projects. The 'Stakeholder' group will be interested parties who will be kept informed of the activities of PCGIN, and from which new project participants will be drawn by the formulation of associated projects. The associated projects will be funded from Defra or some other body with related aims. For related projects, the following will be part of the Network: projects commissioned on areas identified as priorities, LINK projects driven by industry that build on the resources generated and other projects that will benefit or have benefit from the resources generated by the Network. The common denominator among all projects, whether core or associated, and participation in the wider stakeholder group will be good communication, so that all interested parties will become aware of PCGIN and what is being achieved therein.

Related EU project
The EU FP6 Integrated Project 'Grain Legumes' was submitted at the time the PCGIN was first proposed, and this is now underway. The Grain Legumes project represents a major investment (14.4M€ from the EC of a 24.8 M€ project) in the development of basic infrastructures for the development of new strategies to improve grain legumes for food and feed. Due to the coordination of this project by JIC, the PCGIN will be well-placed to interact with the EU project, to negotiate access to relevant materials for UK use and to draw on resources developed in other countries. The PCGIN will develop projects geared to the specific needs of the UK, drawing on resources developed elsewhere to accelerate progress and providing added value to UK investment.

Objective 1: Communication and Delivery

To establish effective communication between the major players with interest in the genetic improvement of pulse crops

Central to the mission of the PCGIN will be establishing a platform with a pipeline for delivery of research outputs to breeders and end-users. This mission will be achieved through the high quality of the integration of the activities of PCGIN with the needs of the commercial sector and related public bodies, together with the impact that PCGIN will have on the activities of these groups.

Our first objective is to build on the links formed by the consultation exercise performed for this proposal, and to forge a close working relationship between UK breeders in the vining pea and combining pulse crops with the research base, both basic and applied. Thus we include the partners: Unilever, Advanta, PGRO and NIAB for applied research and John Innes for basic research. Representatives of these five bodies, together with a Defra representative, will comprise the Management Group responsible for the general direction and setting research objectives, with Unilever and Advanta having *ex officio* roles. The management group will set the broad objectives of the Network and will also oversee the relationship between the Network and existing linked projects. The management group itself represents an industrial sector commitment to the Network. This group will enter into discussion with SASA as the body with the relevant UK responsibilities for varietal description and PVR testing under UPOV. The 'Stakeholders' forum will also contribute to the direction of the core project and is needed to guarantee the broadest relevance of the core activities.

Communication will include the establishment of a database of ongoing research in the area of pulse crops especially in the UK, but also at a European level. This will include a project web site for PCGIN, linked to other Defra genetic improvement networks, SASA, PGRO, JIC and NIAB and the European Grain Legume Association web sites, together with the publication of articles in the relevant trade Journals. Costs associated with the development of web-based links are part of the present proposal (see section 23, 10% of a bioinformatics position at JIC); this will involve establishing and running the PCGIN web site and ensuring compatibility and common features of the web site with that of other DEFRA Networks. The set-up costs for this in the first year will be higher than the subsequent maintenance costs.

The organisation of project meetings will be undertaken by the management group. These will be linked to open ‘Interaction Days’, every second year, that will include the displays of genetic resources that will be available for viewing and discussion each season. The data related to pulse crop research will be maintained at JIC with input from the partners and by interaction with the European Grain Legume Association. The PCGIN as an organisation will be a member of this European Network.

The Management of the PCGIN has several layers. Each organisation participating in the PCGIN has its own management structure responsible for employment and management of the individuals concerned together with all relevant health and safety issues. These organisations are also responsible for the Quality Assurance of the data they generate within the PCGIN, as outlined in section 13. The long-term direction of the PCGIN and establishing its evolving remit is the responsibility of the Management Group, as defined above. This group is charged with overseeing coordination within the PCGIN and ensuring effective communication and dissemination through the 'Stakeholders' Forum. The composition of the Management Group has been established by the parties writing this application in consultation with Defra. By common consent, the Management Group may be enlarged according to the needs of the extended network (ie the core activity within the PCGIN plus the associated projects). The management group will meet twice yearly and the 'Stakeholders' Forum will meet once a year, one meeting of which will also be a management group meeting. The 'Stakeholders' forum will thus be established within the first year of the project, most likely by month six, given the connections established by the preparation of this proposal. An early meeting of the Stakeholder's forum will be considered by the management group. The timing and location of this meeting will take into account external factors such as other relevant meetings organised by other Defra Networks or external bodies. No funding has been requested for an early Stakeholder's forum, but if this occurs it will 'bring forward' the planned first year event.

The Stakeholder's Forum:
The Stakeholder's Forum will consist of the project management group, other research workers on pulse crops in the UK and abroad, end users of pulse crops, and users of the research outcomes e.g. grower representatives and agronomists or other research scientists. Stakeholder Forum meetings will be held each year, and one meeting will follow, or be closely linked to, the time of meetings of the project management team. The management team will present results of activities and plans for future activities to the Forum, which will then be able to judge outcomes and objectives against the requirements of plant breeders, researchers, end users, and growers etc. Where necessary, it will set new objectives and steer the direction of new and associated research.

The composition of the Stakeholder Forum will be decided at the start of the PCGIN, and will adapt to include new interests and directions. Two of the consortium members, NIAB and PGRO, have extensive contacts with pulse growers (including the vining pea crop via PGRO), agronomists and end users through their respective memberships schemes. In addition, NIAB has a formal Memorandum of Agreement with Rres, and can access the ARIA membership scheme. NIAB coordinates PGRO levy funded variety evaluation work on peas and *Vicia* beans and, through the activities of the independent Pulse Trials Advisory Committee, makes recommendations on new and improved varieties. NIAB and PGRO are thus well placed to ensure that the Stakeholder Forum represents all relevant parts of the industry, and will reflect the necessary priorities for improved varieties in UK pulse/vining crop production.

The Stakeholder Forum will play a critical part in the technology transfer phases of research outcomes by continually monitoring and informing the research process of the required industry objectives.

The network project consortium is in the process of compiling a list of membership for the Stakeholder Forum, which will be decided in the initial meeting as described above. This is now being considered in advance of the initial meeting. Potential members include the following groups who are either already engaged with the project or who will be approached at the start of the project: These include the University of Wolverhampton (EUFABA project), SASA (DUS work on peas), Wherry's (pulse processors), NFU – grower representative and feed componders – e.g. Dalgety and including CCFRA. UK-based plant breeding programmes at Unilever and Advanta are already strongly engaged with the PCGIN; other breeders with interests in pulse crops and using European breeding programmes material, including Elsoms, Danisco and Cebeco, will be approached.

A measure of the effectiveness of communication within the network will be given by the development of associated projects. Four avenues will be pursued immediately within PCGIN.

1. Through the preparation of this proposal, a CASE studentship, linking Unilever and JIC and focussing on the relationship between *Pisum sativum* and *Pisum abyssinicum,* <u>has been funded</u> by the BBSRC, began in February 2004 and will be associated with the PCGIN. This project will analyse mapping populations generated from crosses between the two independently domesticated *Pisum* species.
2. Further associated projects are in the pipeline, involving CSL,York and CCFRA, Chipping Campden. The CSL project is associated with the EU FP6 project and will build on metabolite profiling expertise developed at CSL, making these techniques available to the PCGIN.
3. The CCFRA project that is being discussed concerns quality attributes in the vining pea crop, drawing on resources developed, or made available, within the PCGIN, and will be associated with objective 6.
4. A LINK project proposal, focussing on disease resistance / tolerance will be submitted by the participants within six months of the start date of the project. Definition of the content, and submission, of this proposal will form part of Objective 1 for the Management group; the LINK programme will be coordinated by Unilever and will operate in two phases: 6-8 months of literature analysis and consultation will identify the most appropriate targets for a second phase of germplasm screening.

Negotiated access to genetic resources (in particular field bean):

The PCGIN has a responsibility to develop an appropriate programme on field bean genetics relevant to UK agriculture. This requires the establishment of resources and expertise that are currently lacking in the UK. Thus the PCGIN will undertake three tasks in the first year.
(1) We will develop Material Transfer Agreements (MTA) with those labs currently involved in field bean genetics in the EU. The purpose of the MTA will be to provide access to mapping populations of faba bean and to integrate marker analysis of these populations. Preliminary negotiations have begun, and the involvement of these projects with the EU Grain Legumes Integrated Project suggests that this will be relatively easy to achieve.
(2) The Stakeholders forum will be asked to inform the PCGIN concerning the main traits for which genetic analysis in field bean is desirable and most likely to be productive. The outcome of this discussion will determine if the PCGIN needs to develop specific mapping populations to enable the relevant trait analysis.

(3) Once genetic resources (mapping populations and markers) have been established as accessible to the PCGIN, and specific trait genetic questions have been formulated, a field bean genetics proposal will be formulated and costed for discussion with Defra. We anticipate that this proposal will be relatively low cost as it will build on resources available within the PCGIN and elsewhere, but will require some personnel and material support at NIAB, PGRO and JIC.

Monitoring
The effectiveness of communication and exploitation of the PCGIN activities needs to be assessed and the programme itself should be subject to review in the light of this assessment. Accordingly the advice of an independent assessment group (IAG) will be sought at approximately month 18. The IAG will comprise at most six persons. The Stakeholder forum will be invited to propose two independent reviewers (perhaps from the forum itself), the PCGIN will propose two persons external to the UK, and Defra will be invited to propose two additional individuals. This group will assess the direction that the PCGIN has taken and its forward plans and will comment on the PCGIN's success (or otherwise) together with an assessment of its forward plans. The PCGIN will table this report together with its comments for assesment by Defra by the end of year 2.

Time frame
This objective is an ongoing activity that has been initiated already by the preparation of this proposal. We intend to hold at least two meetings per year where we hope to recruit further representatives of the end user groups by the end of year 1. One meeting a year will be a 'stakeholder's forum' but the other will be a smaller meeting of the management group. The smaller meeting may include additional participants (from the wider 'stakeholder' group) for communication, establishment or integration of a new activity.

Objective 2: Phenotyping

Phenotypic characterisation of novel legume germplasm

This objective can proceed immediately for *Pisum*, since this is where resources are available. It will extend to *Vicia*, following consultation by and within the Management Group, and will exploit extant UK *Vicia* germplasm, as well as drawing on EU *Vicia* resources.¶ (see below)

Background:
This objective addresses a current mismatch in available pulse crop data. Hitherto phenotypic data has been collected in several ways. Crop performance data have been collected by NIAB (and SASA) while morphological and some physiological data has been collected in association with germplasm resources. Genotypic data has been collected from germplasm accessions and mapping populations. Thus it is only in the case of specific mapping populations that genotypic and phenotypic data have been collected form the same material. Within the core projects of PCGIN, objectives 2, 3 and 5 seek to redress this mismatch by collecting targeted phenotypic data from material that has already been subject to genotypic analysis (Objective 2), while genotypic data will be collected from appropriate material that has already been correlated with extensive crop performance data (Objective 3). Within Objective

2, we will seek to obtain data from material that is not pre-adapted to agricultural practice. For this reason, the collection of these data will be more difficult than for adapted cultivars; nevertheless these data will complement cultivar data, will be associated with genotypic information and will attract attention to new sources of useful genetic variation.

Genetic resources available within breeding programmes are limited. However, many breeders currently recognise the value of 'exotic' germplasm, and the use of molecular marker methods facilitates the incorporation of allelic variation from this material. This interest is reflected in the CASE studentship outlined above (Objective 1 approaches). The purpose of including 'exotic' genetic variation in the breeding gene pool is to introduce genetic determinants that contribute to yield sustainability. The lack of such sustainability is one of the main reasons for non-inclusion of legume cops in rotations and has been highlighted as a priority for all legumes in the breeders' survey reported in this proposal. Improving these characteristics will increase overall biodiversity of crop systems and will widen the genetic diversity of the pulse crops themselves. Prospecting exotic germplasm collection is clearly aimed at long term benefits to gene pools and resources available to breeders.

Experimental plans:
In this objective we plan initially to undertake a phenotypic description of *Pisum* accessions, selected on the basis of genetic marker analysis, as potential sources of novel genetic variation. The methodologies for choosing germplasm will be based on genetic distance measurements (see below) to ensure that the sample does indeed contain genetic maximum diversity. We will initially restrict the number of accessions to be analysed at all sites to a common set of 5 to 10 lines while protocols are established. In the subsequent years, a subset of ca 20 lines from approximately 50 grown at JIC in the first year will be trialled subsequently at all three sites. This scheme is adopted in order to keep the replication size within manageable bounds and recognising the additional difficulties associated with 'exotic' germplasm. We anticipate difficulty in trait measurement in non-adapted germplasm, coupled with the need to revise the experimental design in successive years, that will necessarily increase the amount of work to be done in order to ensure compatibility between data sets. This objective will provide a description of this diverse material in relation to the trait characteristics identified as UK breeders' priorities. Potential contributions will be in poor agronomic genetic backgrounds and so the phenotyping strategies must be established as a first priority.

The novel set of accessions will be selected using standard multivariate, phylogenetic and population genetic approaches and will exploit data previously generated at JIC (projects TEBioDiv – BI04-CT96-0508 and TEGERM – QLK5-CT-2000-01502 and in collaboration between JIC and Dr AJ Flavell at the University of Dundee). The analytical methods will include the generation of similarity / distance matrices using a variety of distance measures (Nei and Li, Jaccard, simple match[6]) and these matrices will be analysed by principal coordinate analysis and by maximum likelihood and parsimony methods for the construction of dendrograms. Population genetic methods will include modelling approaches such as the 'Structure' analysis described by Pritchard[7].
Phenotypic analysis will involve standard character recording tables and will be undertaken at JIC, PGRO and NIAB. Trait correlation analysis will be undertaken

using distance / similarity matrix data and multivariate analysis including correlation and regression analyses. Approximately 800-900 markers per line will be used in these analyses, composed of in the region of 800 SSAP markers, 20 - 50 RBIP markers and fewer SSRs.

The role of phenotyping.
This objective will undertake a systematic phenotypic description of very diverse germplasm, and for this reason is modest in scale. This objective will not of itself generate ‘useful resources for crop genetic improvement’ - this material is already available, and indeed has been accessed to a limited degree by the commercial sector. However, the purpose is to establish conformity of description across different research groups that is essential for their interaction. For example, within the CGAIR CP programme ‘Generation’ on access to genetic resources, coordinated phenotypic description is recognised as a major issue for the uptake and understanding of genetic resources. If there is no common understanding or coordination of phenotypic description within the PCGIN then it will be impossible to widen the genetic resources available for crop improvement.

The tools and populations to be exploited in objective 2 have been developed at JIC, are available to the PCGIN and outside groups, and are not encumbered by any IP arrangements that restrict their use. The JIC germplasm collection includes the *Pisum* collection, which is the most widely used international collection, and *Vicia* species and varieties that will be available to the PCGIN. We do not anticipate the need to obtain germplasm from other centres (beyond the standard collection of Germplasm) but will negotiate access to additional *Vicia faba* material within the EU project, Grain Legumes.

PGRO has a data base containing information on variety trials for *Vicia faba* with several spring field bean varieties. These will be grown at PGRO and NIAB in the first year for preliminary trials of field tolerance for disease and other performance traits. This information will inform the proposed Vicia project submission in year 1.

Time frame:
The project management team will decide on the planning, procedures and protocols to be adopted during the first year of the project. Genotypes will be multiplied in glasshouses (year 1) and then planted in small plots in the field for phenotypic characterisation at JIC, NIAB and PGRO (year 2). Correlation analysis between molecular marker data (where available) and trait characterisation will be undertaken post harvest in year 2, and the characterisation repeated in years 3 and 4.

Vicia germplasm stocks held at JIC as of 4/12/03

Species: Description; Number of accessions	
Vicia faba :	Both horse and tic bean breeders lines and varieties; 150 accessions
Vicia faba :	Landrace selections; 2 accessions
Vicia narbonensis :	Narbon bean; 5 accessions
Vicia sativa :	Wild; 2 accessions
Vicia johannis :	Wild; 2 accessions
Vicia hirsuta	Wild; 1 accessions

Objective 3: Performance

Varietal performance data, across sites and years, are available at NIAB as Recommended List (RL) trials which are available for “data–mining” purposes and these will be used by the management group to select genotypes for further analysis. A large number of crop characters have been recorded together with site characteristics (eg soil type, rotational history, and local meteorological data). A search facility will be developed in order to use the existing data, and identify a set of 50 cultivars (pea and *Vicia*) (O3/1) that are differential for the traits identified in the assessment of breeders’ priorities that are the targets for trait development. Other databases exist in the UK, and permission will be sought to use information from these if appropriate.

A Defra-funded desk study currently under way at NIAB aims to identify sustainability characters in a range of crop plants, including pulses, in relation to Value for Cultivation and Use (VCU trials). Permission will be sought from collaborators on this desk study to use its findings to inform genotype selection. Enquiries by the participants of this proposal indicate that this permission will be forthcoming; see note below.
The purpose of developing RILs in Objective 3 is to provide the material to access genetic variation for traits scored among varieties by NIAB. RILs developed by JIC previously and made available to MAFF- or Defra-funded projects, and RILs developed under the latter, were not generated from such crosses and so cannot provide the information required. The utility of any RI population is to a large extent limited by trait variation evident for the parents.

The selected pea cultivars (O3/2) will be genotyped with approximately 200 markers (O3/3) of which at least 50 will have known genetic map positions. Genotyping of the selected lines will be performed at JIC by a research assistant, using a combination of methods, mainly SSAP, cDNA-AFLP and intron-directed PCR. Marker analysis will be developed for *Vicia* in parallel. The markers generated will be freely available to the PCGIN and outside groups. The three pea cultivars that are maximally informative on the basis of phenotypic data and marker analysis will be selected for the generation of segregating populations. Three recombinant inbred (RI) populations will be generated from all pairwise crosses aiming for 150 RI lines per population. At two generations per year we will be at F_6 by year 4 (O3/4); a further year of propagation will be needed to reach F_8 where phenotypic analysis can be performed. These materials will constitute an essential resource for the genetic analysis of performance traits that are limiting the current UK pulse crops, principally yield components. Generating this type of genetic resource must be a key function of PCGIN. The details of the development of *Vicia* crosses cannot be specified in advance of the early data-mining exercise.

Note: Data gathered through Recommended List trials, in the most recent past, is funded by the PGRO Levy and, as PGRO is a direct participant in the project, these data will be freely available to the PCGIN. With respect to the National List trials data, the PCGIN will have unfettered access to the summary data subject to permission from Defra, and this will be requested by the PCGIN. Access to more detailed data requires permission from the company that submitted the variety for testing. If on the basis of the summary data we require these data, then the permissions will be sought.

Time frame:
A cultivar set will be identified, based on database mining (6 months); genotyping of 50 selected cultivars with approximately 200 genetic markers will be completed by the end of year 1. Crosses between three maximally informative cultivars will be established during year 2 and the generation of three recombinant inbred populations from all pairwise crosses to F_6 completed by year 4.

Comment on Objectives 2 and 3:
These two objectives are reciprocal. On the one hand, there is an extensive body of marker data for pea germplasm accessions, but this is not coupled to phenotypic data. On the other hand, there are extensive performance data available for legume cultivars that are poorly characterised with genetic markers. Objective 2 aims to add phenotypic data to a carefully selected set of germplasm (defined on the basis of genetic markers). Objective 3 aims to add genetic markers to cultivars with known contrasting phenotypes and to select appropriate parents for the development of recombinant inbred populations for genetic analysis.

Objective 4: Reverse Genetics

Mutant populations are essential for much genetic analysis. An essential component of TILLING (Targetted Induced Local Lesions IN Genomes) is a mutant population to enable targeted searches for mutants in defined genes, for which some sequence information is available. Access to such a pea population within the EU 'Grain Legumes' project will enable a reverse genetic approach to identifying plants that carry mutations in specified genes, and will provide a tool for assessing the impact of candidate genes that may regulate specified traits. We will search for mutants in a wide range of genes (candidate genes) implicated in traits identified as breeders' priorities. The initial focus will be on genes which affect plant architecture, as related to standing ability, and genes that are likely to interact with these genes, as predicted from the wider literature; searches will also include 'architectural' genes implicated in disease (host-pathogen) responses. A similar allele discovery approach could also be applied to targeted searches for novel alleles within germplasm collections.

The EU project will provide a TILLING platform, consisting of an EMS mutant population (one has already developed at INRA, Versailles, France), DNA preparations from pools of mutant plants, a service to assist with mutation screens, and some EST sequence data. During the preparation of this proposal we envisaged that the PCGIN would contribute directly to this international effort in the generation and operation of this legume TILLING platform and the provision of DNA sequence for the generation of a pea DNA array for expression and other studies. These sequences have been made available by JIC and other EU Grain Legume IP participants, so the PCGIN will have no input to these resources. Primers will be generated, based on the annotation, and database screening, of the cDNAs (ESTs).

The TILLING mutant population will be screened for potential mutants affecting plant architecture, specifically genes that regulate the development of the aerial part of the plant, for three main reasons.[¶ (see below)]

- First, these genes are a high priority in the assessment of breeders' needs as the genes selected are likely to determine one or more of: meristem fate, branching habit, flowering time (node to flower initiation), leaf/flower form, and thus influence or control one or more of: yield stability, standing ability, plant architecture as related to weed control and harvest efficiency, nutrient use efficiency by influencing the nature of the intermediate nitrogen reserve[§(see below)], with possible pleiotropic effects on disease control.
- Secondly, these genes are likely to be components of well-characterised genetic regulatory pathways in model systems. Thus candidate genes that are likely to have interesting consequences for breeders can be identified.
- Thirdly, the consequence of mutation in these genes can be assayed relatively easily at the phenotypic level as well as in terms of genetic interaction with previously characterised mutants, that are additional tools for the molecular analysis of the mutant phenotype.

Thus this choice of target genes presents an optimal combination of likely impact.

¶ The trait selected for TILLING (plant architecture) is a complex interaction of many genes, but in pea many of these genes are well characterised; to date 11 such genes have been cloned and characterised in pea and of these JIC has been involved in the isolation of six. There are many candidate genes that are well characterised in other systems and which are expected to have predictable interactions with genes already characterised. Thus plant architecture in pea is well in advance of trait genetic approach such as QTL analysis. This is in marked contrast with traits such as protein quality or disease resistance where (with the exception of the trypsin inhibitor or one virus resistance locus) the underlying genetics is poorly understood. Our focus on a trait under simple genetic control is in part determined by the genetic tractability of the problem and in part by the practical problems associated with plant architecture in the pea crop.
It should be emphasised that the TILLING resource that will be developed within the EU project will not be available for some time to come. The PCGIN needs to set priorities to finance screens of this population, and the EU project through its technology transfer platform will provide the route that the PCGIN can use for the benefit of its stakeholder community.

§ Comments on the relationship between plant architecture and seed filling:
Source: Dr C. Salon, INRA, Dijon.
1) Photosynthetic activity depends upon intercepted radiation and will condition C flux towards seeds during the seed filling period and so influence the duration of seed filling and seed size.
2) Photosynthetic activity depends upon the amount of N per unit surface area of leaves which diminishes throughout the growth cycle for all foliar strata as sink (seed) demand drains N storage within leaves: remobilisation of leaf N homogenises N distribution within the plant (sunflower: Connor *et al*., 1995[8]; soybean: Grandigard, 2002 [9]).
3) An optimal CO_2 acquisition by the canopy can be obtained only if N distribution allows optimum PAR utilisation at each stratum (Shiraiwa and Sinclair, 1993 [10]). The amount of N per surface area of leaves can be related to light distribution and N status of plants. SLN (N per surface area of leaves) is conditioned by PAR distribution: (peanut: Wright and Hammer, 1994 [11]; wheat: Bindraban, 1999 [12]) and is confirmed by numerous studies (wheat: Goudriaan, 1995 [13]; Dreccer *et al*., 2000 [14]; cotton: Milroy *et al*., 2001 [15]).
4) The heterogenous N partitioning within the various foliar strata may influence N allocation to seeds (Mooney and Gulman, 1979 [16], Shiraiwa and Sinclair, 1993 [10] and Lemaire et al., 1991 [17]) although, for soybean, N in the plant seems to represent a common pool available for all seeds in the canopy (Grandgirard, 2002 [9]).
As a conclusion : Architecture influences not only leaf position (and so N distribution within canopy) but also may influence the leaf /stem ratios: as their N content differs and is differentially mobilized during seed filling [C. Salon commented: “N in stems is solicited later in the growth cycle”], plants having different architecture may therefore have different remobilisation efficiencies towards seeds, different light interception efficiencies (and hence C capture and seed filling duration), as well as differences in the amount of N remaining in the plant at maturity.

Time frame:
relevant sequencing data will be available by month 18, database work and primer design completed by end of Year 2 and the PCR-based TILLING screens will be in years 3 and 4.

Objective 5: Genetic Mapping in crop legumes
5a Provide novel germplasm for trait analysis (Fast Neutron mutant analysis and mapping)

Objective 5a will provide a systematic collection of deletion mutants as a new genetic resource to be entered into the public collection. Similar resources are available to cereal geneticists and breeders but this resource will be entirely new for legumes. A population of ca 9000 fast neutron (FN) irradiated pea lines in a well-characterised genetic background has already been developed at JIC and, as these are characterised, they will be included in the JIC germplasm collection and so made generally available. A comprehensive set of deletion lines, corresponding to an ordered set of deletions where each line eliminates a small section (or set of sections) of the genome but where collectively these cover the whole genome, is beyond the current scope of the PCGIN, but we propose to initiate the process of the characterisation of such a resource and will determine its general utility, as a first step towards having complete genome coverage.

From an initial survey of the first generation selfed progeny of ca 3000 of this population, it is clear that multiple mutants of equivalent phenotype have been recovered, based on allelism tests. This suggests that this population has good genome coverage with deletion of most chromosomal segments that do not give rise to homozygous lethal mutations. Mapping a substantial number of the deletions (currently estimated at approximately 1000, but the number depends to some extent on the average size of deletion) would provide a resource constituting an ordered set of segmental losses. These lines would include null mutations for many genes and gene families and provide an important resource in relating quantitative trait locus (QTL) position to individual genes. That is, once a QTL has been located to a given genomic segment, the 'deletion library' can be accessed to determine which deletions cover the QTL interval. These deletion mutants can then be classified according to the QTL phenotype, and overlapping deletions will specify the gene content to be investigated using comparative genetics and TILLING approaches.

Within PCGIN, we propose to determine the average size of deleted segments and a set of 12 independent M_3 individuals will be selected for marker and map analysis. cDNA will be generated from young seedlings and used as a template for AFLP analysis. We can examine all *Pst* I/*Mse* primer combinations with a suitable degree of selection using 256 PCRs, corresponding to approximately 100 duplicated gels with 15,000 transcribed gene fragments resolved per individual. This constitutes a reasonably extensive gene search, although it is by no means complete. Using this analytical approach, we can determine, with reasonable accuracy, the number of genes deleted per FN line. The cDNA-AFLP fragments corresponding to the missing transcripts will be sequenced and the genes identified and mapped. Sequencing costs are required for the characterisation of cDNA-AFLP fragments. Sequencing will be performed using standard protocols and has been costed within this proposal. The sequencing will be put to tender; we anticipate using the JIC Genome Centre.

These procedures and methodologies, which are technically demanding, will be undertaken by a research assistant {TBA} working in close association with L. Turner who has relevant experience.

The fast neutron population is a resource that has been developed, and the purpose of the activities proposed within objective 5b is to assess the potential of this resource for trait genetic analysis. The intention is to ask whether it is feasible to develop an ordered set of deletion mutants covering the pea genome. In effect this part of the project is a cost/benefit analysis, and it will deliver a clear strategy with respect to the use of deletion mutants in ‘mendelizing’ QTLs in a way that facilitates gene isolation. Such a development would be a large scale activity and it is not envisaged that this would be completed within the PCGIN. However if such a resource development is feasible this would provide a resource for genetic analysis that would tie trait determinants to candidate genes by simple genetics. Such a resource would be easy to use and accessible to a classical genetics project but tied to genome sequence information via the identity of the deletion mutants held as a genetic resource. The PCGIN objective is to test whether this approach is practicable. The predicted output of the work is the provision of novel mutant germplasm, together with detailed analysis of selected lines.

Time scale:
This pilot study for the generation of a systematic gene knock-out panel for trait analysis will begin in month six and will be completed by the end of year 2.

5b Integrated Genetic Maps

A first priority of objective 5b (Integrated Genetic Maps) is the establishment of the relevant segregating populations within PCGIN. This needs coordination with currently funded projects (within the EU, in particular EU-FABA and 'Grain Legumes', or outside the EU) to obtain access to mapping populations of *Vicia* (and lupin) that are relevant for the study of genes controlling UK priority traits. This negotiation with external collaborators will be undertaken by Dr N. Ellis. Links with the University of Reading will be established for lupin (see page 5). Where appropriate crosses cannot be accessed external to the PCGIN, they will be generated by JIC. The development costs associated with this will come partly from JIC and from PCGIN (glasshouse use costs and person time to perform crosses and manage the populations). The relevant pea lines and crosses already exist at JIC.

Within the first six months of the project, the most effective strategy for relating the genetic maps of pea, field bean and lupin will be identified. This requires, in addition to the relevant mapping populations, a set of gene-based markers that can be screened using appropriate primers by PCR. A set of 100 PCR gene intron-based markers that can be placed as markers on the populations (extant or to be generated) will be defined. It is anticipated that these will detect largely single nucleotide poymorphisms (SNPs) or additional small alterations within gene sequences. Genetic mapping of these markers will allow the definition of molecular markers for mapped priority traits that can be exploited by breeders.

Time scale:
A strategy for relating the genetic maps of pea, field bean and lupin will be defined by month 6, based on consultation and coordination with currently funded projects within the EU (Grain Legumes, EU-FABA) and internationally. Appropriate segregating populations will be established (or initiated, where necessary) within the PCGIN by the end of year 1. By the end of year 2, a set of 100 PCR gene-based markers that can be placed on the populations from all species will be defined. Genetic mapping of these markers will proceed during Years 3 and 4; integration of maps for priority traits will be performed by the end of Year 4.

Objective 6: Genetics of Seed Quality Traits

The performance traits identified by breeders are addressed in Objectives 2 - 5. Within Objective 1, we will extend the range of stakeholders to include crop end users. We anticipate that this group's interests will focus on seed quality traits and expect that the first 'stakeholders' forum will flush new ideas and assist with prioritisation. Within the breeders' survey conducted as part of this proposal, it is noteworthy that quality traits ranked 'High' for vining peas, but not for combined crops. The reason for this is simply that improved quality in legume seed for animal feed does not have a return to farmers or to growers, as a consequence of the economics of seed formulation. This may change if the market is diversified so that quality differences match the needs of different species (e.g. chicken versus pig). The lack of linkage between crop quality and crop usage is a major disincentive to the development of this market. Markets for legume seed products exist within the UK, that are currently relatively minor but that nevertheless do not use home-grown raw materials. These markets include end-uses such as in the formulation of vegetarian food products and non-allergenic plant milk substitutes. Furthermore, there is growing interest in exploiting seed products from sustainable crops for the manufacture of biofuel.

Within PCGIN, the genetics of seed products and development of associated markers for breeders' use must focus on products deemed by industrial end-users to be of added value. Tools for the genetic analysis of these traits need to be developed as a basic infrastructure. We propose to develop and analyse recombinant inbred populations that segregate for key traits in relation to seed and protein quality, as defined by these end users, building where appropriate on QTL analyses already undertaken in relation to protein content and quality. The development of these basic infrastructural resources tailored to end users' needs will enable the development of related satellite projects.
QTL analysis will involve several methods (QTL Cafe and JoinMap 4 QTL). Costs associated with a licence for the latter programme, a PC and the costs for M Knox, who will oversee these analyses for the PCGIN, to attend a relevant training course in Wageningen are included here.

Time scale:
Identify quality trait targets defined by UK industries (as part of first stakeholders forum) by the end of year 1. [i.e. we will prioritise traits based on end-user consultations].
Development of assays for these traits and initiate the generation of recombinant inbred populations during year 2. [Without an assay the traits cannot be assessed For some of these traits, assays may already exist but, if not, they will need to be developed. Where assays do exist, they may be cumbersome and are likely to require modification to facilitate high-throughput genetic screening. Protocol development and adoption are milestone O6/2].
Preliminary genetic analysis (at F_6) of the genetics of these traits in a low density genetic map by the end of Year 4.

Novel germplasm and other resources generated by PCGIN
An early objective of the management group of PCGIN will be to agree a process for the release of new germplasm and other resources to the commercial sector. This mechanism will need to be compatible with the obligations placed on PRSEs to seek commercial advantage from government investment in research while maximising the prospect of practical uptake towards achievement of government’s priorities for sustainable agriculture.

The PCGIN will not generate resources based on materials and knowledge with use restrictions that would limit uptake by the research community and industry. At the outset, the genetic resources placed at the disposal of the PCGIN or the projects that are under the “PCGIN umbrella” will be identified, including those already in the public sector. Once this is agreed, a cash value can be placed on any materials (germplasm, markers etc.) transferred from the “greater” PCGIN (as defined above) to commercial end-users. In practice, we anticipate most of this will be data made available publicly but, where the information is not fully in the public domain, a standard royalty agreement associated with the exploitation of these resources will be established. Any income received will be allocated to supplement and maintain the activities of PCGIN, as it is likely that this proposal would be favoured by breeding companies who will see their revenue being recycled to their continuing benefit.

Tools provided by the PCGIN

The function of the PCGIN is to provide the genetic resources and know-how that the combinable crop breeding industry and the vining pea breeding industry need for their breeding objectives. The project as described identifies the need for several RIL populations to be developed (in Objectives 3 and 6) that will be required for trait dissection. The proposal does not define the traits to be examined in these populations because that requires input from the stakeholder community. The timescale for the development of these resources is long with respect to the duration of the project, but we anticipate these RILs to be a lasting resource that the stakeholder community will be able to analyse beyond the timescale of the present proposal. The RIL populations are therefore a genetic resource that will be available to the stakeholder community in perpetuity.

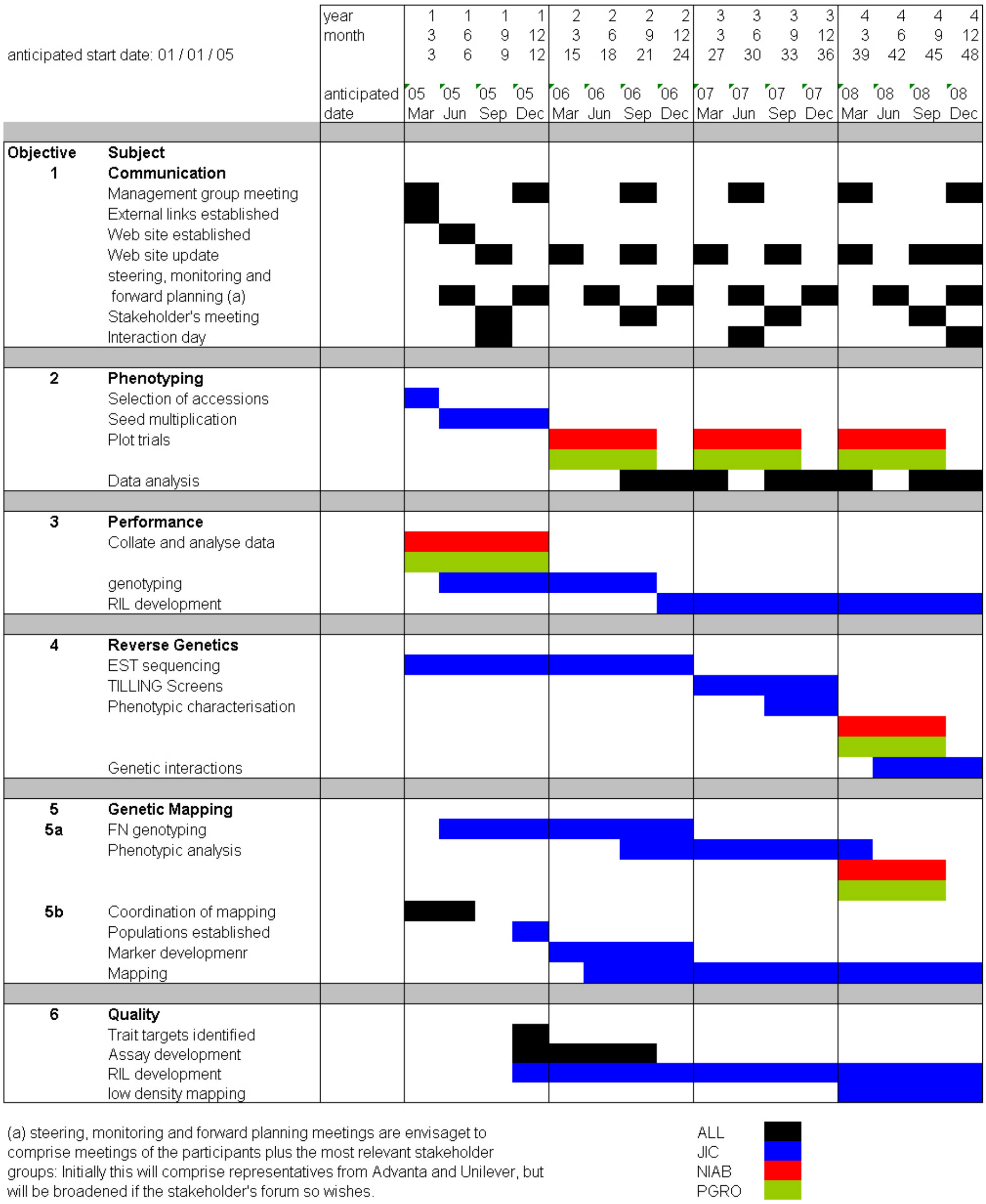
The traits to be analysed will be defined to a large degree by the stakeholders, and the marker methods used for the analysis of these RILs will be the most cost effective available, but once trait - marker associations are established it may be necessary to customise these further for low cost high throughput activities by the commercial organisations. The extent to which the PCGIN can provide a technical resource for the UK pulse (and vining pea) breeding community will be a subject for the stakeholder’s forum. Ultimately we should envisage the relationship between the research providers within the PCGIN and end-users to be such that training, and hands-on experience, in the use of marker technology and screening would be provided; however we recognise that it may be more cost effective to centralise such resources. These policy decisions by the stakeholder group cannot therefore be presented within the PCGIN proposal and require extended discussion within the stakeholder forum.

Species

This proposal concerns three species: pea, field bean and lupin, but we recognise that there is legitimate interest in other species (*Phaseolus* for example). Similarly forage legume genetics and breeding could benefit and mechanisms to include these groups will be sought. We recognise that the specific tasks identified in this proposal have a strong bias to pea, reflecting the previous investment in this species. However the PCGIN will prepare a specific proposal for stakeholder-focussed genetics in field bean by the end of its first year. For lupin, our plans are less clear and will depend to some degree on interaction with lupin geneticists and breeders outside the UK.

The strategy of the PCGIN is to build resources that are stable: RILs that will be incorporated in public germplasm collections, and marker and trait data associated with curated germplsam, cultivars, RILs and exotic germplasm. These then will be directly available to stakeholders independent of future funding sources. In addition to this, the aim of the PCGIN is to provide a resource of expertise that the stakeholders can draw upon either collectively or individually.

Time lines for main activities:



Comments:
Objective 1
“External links established” includes the links between the PCGIN and related international projects as well as the links to the Stakeholder's forum and the other Defra Networks. The dates of website updates are formal reviews of content with associated updates, we expect additional updates as relevant information becomes available.
Objective 2
The three years of plot trials are needed in order to obtain good replication. We anticipate that the first year of trials will provide some information as well as troubleshooting our methodology while the two remaining years are required for good replication.

Milestones

Milestone	due date	Description
O1 / 01	m 3	External links to EU and CGAIR projects established
O1 / 02	m 6	Web site established and integrated with other Crop Improvement Networks
O1 / 03	m12	First Stakeholder's meeting (note: this will be as soon as is feasible, before m12 at the latest, and will be repeated annually)
O1 / 04	m12	First Interaction Day
O1 / 05	m36	Second Interaction Day
O1 / 06	m 9	Strategy to underpin genetic improvement in *Vicia faba* agreed and available resources identified
O2 / 01	m 3	Selection of the 20 diverse accessions, with details at least 150 SSAP markers per accession
O2 / 02	m 9	Protocols for phenotype evaluation (see ¶ below)
O2 / 03	m12	Seed available for plot trials
O2 / 04	m24	First Plot Trial completed and data available
O2 / 05	m36	Second Plot Trial completed and data available
O2 / 06	m42	Completed analysis of plot trials
O3 / 01	m 9	Performance data collated (see § below)
O3 / 02	m21	Selection of maximally informative cultivars subset for genotyping
O3 / 03	m21	Genotyping completed (50 lines with 200 markers) and selection of parents
O3 / 04	m48	RIL populations at F_6 or later generation
O4 / 01	m24	5000 EST sequences available (negotiated the EU project ‘Grain Legumes’)
O4 / 02	m36	First TILLING screens completed
O4 / 03	m48	Phenotypic assessment of mutants selected by reverse genetic screen
O5 / 01	m24	Maps for 12 FN deletion lines completed: deletions located
O5 / 02	m36	Preliminary phenotypic assessment of deletion lines
O5 / 03	m45	Plot trial data for FN deletion lines
O5 / 04	m12	Mapping populations and markers established for pea, field bean and lupin (see † below)
O5 / 05	m48	comparative map data sets available
O6 / 01	m9	Quality trait target defined
O6 / 02	m15	Quality traits assay defined
O6 / 03	m48	Genetic analysis of quality traits at F_6 generation

¶	The protocols are the methods by which the data is to be collected including replication and block design as well as measurement procedure.
§	The intention is that the data would be collated by month nine and analysed by month 10
	Pea mapping populations are already available, but some additional populations will be generated or obtained as described in the text. O5/ 04 depends on activity external to the project, if not successful by Month 12 then SO5/ 06 is substituted.

Secondary Milestones:

Milestone	due date	Description
SO1/06 SO1/07 SO1/08	Months 24, 36 & 48	Stakeholder's meeting (one per year by target date early summer anticipated)
SO2/06	Month 39	Revised phenotyping plan for third trial
SO2/07	Month 30	Initial analysis of first plot trial
SO3/03	Month 33	F_2 of selected performance trait crosses
SO3/04	Month 48	F_6 seed from performance trait crosses
SO5/06	Month 12	If O5/ 04 is not completed alternative strategy in place

Quality Assurance

JIC QA policy has recently been revised and is outlined below; however all laboratory work is subject to COSHH regulations, is subject to inspection by safety committees and conforms to GLP. All work involving GMOs and GMMs is regulated by the duly constituted Biological Safety Committee and reviewed by HSE and DEFRA when appropriate. This work all corresponds to the EU directive 90/219/EEC as amended by 98/81/EC

All laboratory work is subject to scrutiny by project leaders to assure accuracy and appropriateness of interpretation. Relating to both Q. 13 and 14, appropriate statistical methods required for the PCGIN fall into three areas: genetic diversity analysis, genetic mapping, and standard statistical tests. For the first of these three, we are directly connected to a BBSRC-funded bioinformatics project at the University of Dundee and through the John Innes Computational Biology Unit. These two sources provide methods that are directly tailored to data generated by the Network and to the analysis of these specific data types.

For genetic mapping, the JIC Crop Genetics Department is a major source of expertise in this area, and we have sought support for the appropriate licence upgrades and up-to-date refresher courses for current software.

For standard statistical problems the JIC has a regular 'helpdesk' run in conjunction with Dr Roger Stern and associated staff of the Statistical Services Centre, University of Reading, who also run regular courses at JIC on data analysis and optimising experimental design.

NIAB - NIAB is currently working towards full ISO 9001:2000 accreditation for 2003, which will govern all laboratory, field, and administration operations. In addition, analytical laboratories operate according to the principles of GLP. NIAB is a GEP (Good Efficacy Practice) registered with PSD, and uses the principles of this system to govern field work. Seed testing systems are accredited by ISTA.

PGRO is supported through the Pulse Levy, and together with NIAB produces the UK recommended list of pea and bean varieties. PGRO is accredited by NIAB and UKASTA to carry out seed tests for pathogens and pests of pulse seeds and is officially recognised by PSD for efficacy testing of pesticides.

Statistical Input:

Appropriate statistical methods required for the PCGIN fall into three areas: genetic diversity analysis, genetic mapping, and standard statistical tests.

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Note no guidance has yet been sought on establishing the field trials protocols as this corresponds to milestone O2/02