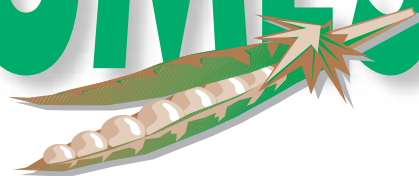


# GRAIN LEGUMES



AEP

The magazine of the European Association for Grain Legume Research  
Le magazine de l'Association Européenne de recherche sur les Protéagineux

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*GERMINATE: toutes les données sur le germplasma*

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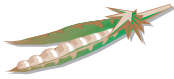


**Food uses and health benefits of lupins**

*Lupin: atouts santé et produits agro-alimentaires*

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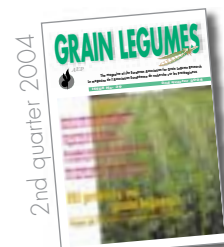
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## Modern and healthy food?

Although grain legumes are common in Middle-Eastern, Latin American and Asian diets, EU pulse consumption has decreased markedly in recent decades with only a minor comeback recently with the popularity of natural food and vegetarian cuisine.

Yet, these grains are a perfect nutritive complement to cereals and there is scientific evidence that legume seeds and related components have a positive role to play in the prevention of chronic diseases.

Should we learn to use exotic recipes, modernise those used by our grandmothers or create new ones in order to reintroduce legume grains in our daily meals? Or should we develop innovative foods that contain home-produced plant-based ingredients included during modern food production procedures? In fact grain legumes are versatile: a source of whole seeds, organic food items and vegetable ingredients.

The Healthy-Profood project has assessed the feasibility of using lupin seeds to produce healthy foods. The possibility that lupin products could provide an alternative to soya products is the topic of the special report in this issue.

We encourage you to join us at the final conference of HEALTHY-PROFOOD (free entry) in Milan in November to obtain further details.

**Anne SCHNEIDER**  
Managing Editor

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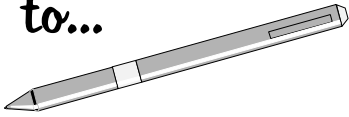
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Carte blanche  
to...



Peter Watts\*

## Canada's pulse industry targets North American food market

Canada is one of the world's largest producers and exporters of grain legumes, also known as pulses, or more specifically peas, lentils, beans and chickpeas. In 2004, pulse production reached a record 4.5 million tonnes (about 8% of global production) or 7% of arable crop production in Canada. Last year's crop included 3.3 million tonnes of peas, 960,000 tonnes of lentils, 220,000 tonnes of dry beans and 51,000 tonnes of chickpeas.

As for many other crops, Canada relies heavily on export markets to sell pulses. On average, around 70% of Canada's pulse crops are sold off shore each year. But given relatively low returns from the world market in recent years, Canada's pulse growers have begun to look at opportunities in their own back yard. North America provides a large, potentially high-value market with ample room for growth in value-added food and industrial products derived from pulses.

To translate opportunity into real outlets for their products, Canada's producers have started to allocate more research dollars toward quality and utilisation to gain a better understanding of the functional properties of pulses, their potential applications as well as their nutritional benefits. The increased focus on utilisation by the pulse industry will complement and strengthen existing research being conducted by the scientific community and food industry.

The growing need for more value-added opportunities for Canadian pulse crops was given a boost in the spring of 2005 with funding from Agriculture and Agri-Food Canada to support innovation in product development, focusing on opportunities in North America's food markets. The new initiative, dubbed the 'Pulse Innovation Project', will have its headquarters at Pulse Canada – Canada's national voice for pulse growers based in Winnipeg.

The project will support the development and commercialisation of pulse foods that are economic, convenient and enhance nutrition and health. The Pulse Innovation Project will receive broad input from the scientific community and food processing industry in the development of a strategic approach to the expansion of markets for peas, lentils, beans and chickpeas in North America. For more information on the Pulse Innovation Project, log onto the Pulse Canada web site at [www.pulsecanada.com](http://www.pulsecanada.com). ■

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## GL-TTP: a new technology transfer platform for the international grain legume community

GL-TTP is an international Technology Transfer Platform created by the Grain Legumes Integrated Project primarily to work at the integration, assessment and commercial exploitation of the results of GLIP research. GL-TTP will facilitate the transfer of knowledge and state-of-the-art technologies between research and industry in order to boost the production of grain legumes.

The mission of GL-TTP is to provide enabling tools in order to:

1. increase grain legume production through the use of more robust varieties and improved crop management,
2. improve and diversify grain legume products for the feed and food industry through better grain quality and new processing techniques.

GL-TTP will initially focus on the interaction between research scientists and plant breeders in order to facilitate and expedite the genetic improvement of grain legume varieties to suit the needs expressed by the grain legume producers and end-users.

The privileged access of GL-TTP to the molecular tools and discoveries generated by GLIP will directly benefit the members of the platform. Notably, GL-TTP will distribute and adapt genomic technologies to:

1. characterise the genetic diversity kept in germplasm banks, so that plant breeders can choose genetic resources based on defined genetic criteria to enlarge the genetic basis of their breeding pools and introduce new genetically-defined traits of interest,
2. generate targeted genetic diversity for breeders when available genetic resources are too narrow (this can be done without the controversial use of Genetically Modified Organisms),
3. identify new genes potentially involved in a trait of agronomic interest thanks to sequencing projects and expression profiling studies,
4. validate the function of those candidate genes using high-throughput functional screens,
5. generate molecular markers, or 'tags' to these genes, so that plant breeders can use high-throughput genomic screens to select for superior plants.

For each technology transfer, GL-TTP will provide technical support. Workshops are in the pipeline for plant breeders eager to learn new technologies and how to exploit the wealth of genomic data publicly available on the internet. GL-TTP will also provide its members with assistance for the integration of marker-assisted selection in their breeding programmes.

Importantly, GL-TTP will initiate a European research project to investigate the cost-effectiveness of marker-assisted selection, from the field to the lab and back to the field, so that plant breeders are ensured of the economic relevance of including molecular techniques in their breeding strategy.

Upon multi-disciplinary surveys, GL-TTP will develop an integrated grain legume database that will be instrumental in the definition of technology transfer programmes and useful as a source of information for scientists and industrialists.

GL-TTP is set up to work in close collaboration with AEP. Consequently, GL-TTP will benefit from the network of the international grain legume community developed by AEP during the last twelve years. AEP and GL-TTP will share strength to animate grain legume research throughout the network. GL-TTP will complement AEP by facilitating the exploitation of the results of grain legume research by industrialists.

GL-TTP adopts a strategy to stimulate cooperation between grain legume organisations so that services and technology transfers can be set up more efficiently and at reduced costs for individual actors at every step of the transfer.

GL-TTP is a not-for-profit association under the French law, so-called 'Association loi 1901'. The detailed structure and organisation of GL-TTP is described in its official statutes, available on demand at [gl-ttp@prolea.com](mailto:gl-ttp@prolea.com).

Until elections take place at the first General Assembly in Montpellier in February 2006, the GL-TTP Council and Executive Committee will be composed of the eleven founding members of GL-TTP. After the General Assembly, the elected GL-TTP Council will be composed of two balanced sections that will represent the interests of science and industry with equal weight. In this way, the Council will orient the activities of GL-TTP according to the wishes of both research scientists and industrialists.

Membership is now open to research organisations and grain legume industries that want to contribute to, and benefit from, the international network of the GL-TTP. More information on the GL-TTP and how to become a GL-TTP member is available at [www.grainlegumes.com/gl-ttp/](http://www.grainlegumes.com/gl-ttp/). ■

Source: Catherine Golstein, GL-TTP Scientific Manager.  
Email: [c.golstein@prolea.com](mailto:c.golstein@prolea.com)

### Final conference of the Healthy-ProFood project, 9–10 November 2005



At this conference, entitled 'Healthy and added value food ingredients from lupin seed' to be held in the prestigious Visconti Palace in Milan, delegates will hear from leading experts on advances in a number of related topics: new processes for preparing lupin protein ingredients with improved technological and sensory characteristics; the nutritional characteristics of lupin protein; new lupin protein-based food items; novel analytical tools for food quality assessment and traceability; potential health benefits of lupin food ingredients/items in hypercholesterolaemia, hypertension and diabetes; and possible risks of allergy. It will also be an opportunity to meet with academics and researchers from around the world and participate in interdisciplinary discussions and share ideas at both the conceptual and applied level. Following the Opening ceremony, there will be twenty-two short presentations, followed by round table discussions on different issues relevant to the future of lupin in human nutrition. Lupins for food and health are also the subject of our special report in this issue of *Grain Legumes*.

Source: A. Arnoldi, DISMA, Italy. Email: [anna.arnoldi@unimi.it](mailto:anna.arnoldi@unimi.it)



# PCGIN: a new UK national project



The Defra<sup>1</sup> Pulse Crop Genetic Improvement Network (PCGIN), which started with effect from 1 April 2005, will establish a platform that serves the process of legume crop improvement in the UK. It will:

- 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,
- 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,
- 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,
- 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.

During the negotiation phase, commercial input and consultation with the broader legume breeding community led to a set of priority traits that are to be the focus of the genetic improvement strategies for pulses. The results of this consultation highlight the major obstacles to increased pulse production in the UK and, by inference, priorities for research.

## Primary objectives and core scientific activities

The Network has six inter-related 'core' objectives:

**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.

**2. 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.

**3. Performance\*** data for modern cultivars are available but this material has never been genotyped extensively. 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.

**4. 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.

**5. 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 (EUFABA) 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.

**6. 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.

## 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; in particular, the coordination of GLIP by JIC will mean that the PCGIN will be well-placed to interact with the EU project. ■

<sup>1</sup>Department for Environment Food and Rural Affairs, UK. Source and further information: <http://www.pcgin.org>



## Toledo 2004: nutritionally active factors in legume seeds and oilseeds



The 4th International Workshop of Antinutritional Factors (ANFs) in Legume Seeds and Oilseeds took place in Toledo, Spain on 8–10 March 2004, with notable success. This was its first time outside Wageningen. This conference signalled a major shift in our perception of the role of ANFs in human and animal nutrition and health. The main conclusion singled out from the workshop sessions was the recognition that the naturally occurring ANFs in legume seeds and oilseeds, have a major biological role that is not always harmful to health but could also be beneficial. Thus, the participants of all four discussion groups recommended that instead of antinutritional factors these materials should be called nutritionally active factors (NAFs) or biologically active substances (BASs) or compounds (BACs) or just simply functional factors (FFs).

### Different analytical methods

Discussion Group 1 showed that despite recent advances in methodologies there are still outstanding problems in the analysis of ANFs. Although a chemically diverse group with diverse modes of action, ANFs are currently grouped together. Actually, each group requires a different analytical approach. Thus, more research is needed to develop new and rapid, but reliable methods for the analysis of protease inhibitors for the food industry and non-destructive methods, such as Near Infrared (NIR) methods, to aid breeding programmes. The involvement of EU networks for exchanging samples of seed (for example, oilseed rape and lupins) between different laboratories, and for the standardisation of analytical techniques would be a great benefit to all.

### Standardisation of protocols

Group 2 concentrated on the nutritional role(s) of ANFs. For this the individual ANFs must be purified before their physiological effects, either singly or in interactions with other ANFs or nutrients in the diet, can be identified. For comparability the animal study protocols need to be standardised. A precondition of this is to use properly formulated purified and/or neutral diets, into which the feed ingredients and/or ANFs can be incorporated. It is also essential, depending on the aim of the studies, to use young, rapidly growing (preferably home-bred animals) spf (special pathogen-free, parasite-free) or fully-grown adult laboratory animals with the same weight. To establish their physiological effects, the most important variables are the ANF dose and the time of exposure (dose  $\times$  time) and these studies should in the first instance concentrate on the physiology and bacterial ecology of the gastrointestinal tract, with particular attention to possible probiotic effects on gut bacteria and the modulation of the systemic- and mucosal-immune systems, specific immune organs of the body, the spleen, bursa of Fabricius and thymus. Effects on the local (gut) and peripheral endocrine systems, the nervous system and other organs and communications between these can be determined when the observed physiological effects

of ANFs are understood better, but all these effects need to be established accurately. The scope of the problems that ANFs may present to consumers has increased dramatically with the cultivation and commercialisation of genetically modified (GM) crops and organisms (GMOs). There is a need to develop *in vivo* and *in vitro* risk-assessment methods that address all the safety problems with these crops and new high-powered techniques to identify the appearance of unpredicted and unintended toxins and allergens.

### ANFs and human nutrition

Group 3 addressed the mode of action and the effects of ANFs in human nutrition, with special emphasis on the achievements of the 'Network for Nutrition in Spain' and the 'Spanish Legume Association'. A number of research tasks that still needs addressing includes determining whether purified or matrix-bound ANFs will behave differently in the gut, particularly as *in vivo* systems are more complex than any *in vitro* simulation, and whether processing will change the mode of action of ANFs. Differences in the species, age and sex of animals can also have major effects, and results of animal experiments can not always be extrapolated to humans. The dose, the duration of exposure, the possibility of accumulation by the body or breakdown generating more toxic products, are all factors of importance for ANF actions. It has to be recognised that changes in the ANF content of a plant by breeding may affect the viability, yield, pest-resistance, etc of the plant and such changes may override any potential benefits of the modification (such as GMOs) in nutrition. Health authorities are responsible for advising consumers about the potential risks of some ANFs by labelling, particularly of known allergenic ANFs, and providing instructions about how best to prepare and cook legume-based foods to optimise their nutritional value and remove the harmful effects of ANFs. It will also be necessary to update the book on legume ANFs issued by FAO and establish a standardised EU data bank system on the content and composition of ANFs in raw and processed legume seeds, oilseeds and foods/feeds for consumer protection. With the inclusion of medical and veterinary scientists, generally acceptable *in vitro* and long-term *in vivo* nutritional and safety testing methods should be developed. Clinical studies with human volunteers and epidemiological studies of human groups consuming legume diets are needed.

### Modifying ANFs

Group 4 discussions focused on the use of biotechnology, plant breeding and processing technologies for the modification





of ANFs. The group could not arrive at a consensus on the issue of genetically modified (GM) plants and particularly the possibly harmful unintended consequences of gene transfer. However, most members recognised its usefulness as a laboratory technique for understanding gene function in plants and identifying unexpected and unintended changes. There was also agreement in the group on the usefulness of marker-assisted selection (MAS) in plant breeding, particularly for the selection of variants rich in natural beneficial factors and screening for mutants containing advantageous genes. The group recommended the setting up of a database of bioactive factors that could be used in MAS both by plant breeders and human/animal nutritionists. The importance of processing in reducing or eliminating allergens and toxins was stressed. Unfortunately, since processing may also generate new allergens and toxins, finding new processing methods may be necessary. A novel recommendation by the group was that an examination of the processing effects should also consider the changes in consumer consumption habits and their reaction to the processed product. This may be particularly important in the light of the ever-increasing problem of human obesity.

### A major milestone

The Toledo ANF Workshop was a major milestone in our understanding of the many roles of ANFs in nutrition and health. The emphasis moved away from the simplistic and mechanistic studies of the past, opening up new approaches by exploring the direct effects of ANFs on cells of the gastrointestinal tract and on the functional consequences of their presence in the diet on metabolism, the immune and endocrine systems, and on the gut bacterial flora. With these new directions we shall also be able to learn how the direct effects of ANFs on the gut can influence the organs of the body and metabolism, and how these may be exploited

### First GLIP dissemination event – Progress in grain legume research for EU agriculture, Madrid, 27 October 2005



Eighteen months after the launch of GLIP, Laurent Bochereau, of the European Commission and responsible for the priority 'Food safety and quality' of the European Union's 6th RTD Framework Programme will open the first one-day dissemination event in Madrid with a presentation on European research programmes related to plant production systems.

This dissemination event with simultaneous English/Spanish translation is organised by AEL (Asociación Española de Leguminosas) and AEP on behalf of the project consortium.

Experts from all over Europe will present the current progress being made in GLIP and other on-going activities with grain legumes. The morning has three sessions: Grain legumes in Europe (two presentations), What benefits for agricultural sustainability (three presentations) and How to enhance grain legumes (two presentations). Following lunch there is a press conference for journalists and a poster session to publicise national and international projects. The final session, What progress in genetics and plant breeding (six presentations) covers disease tolerance and resistance to saline and drought stress, genomic tools, comparative genetics and the newly launched Grain Legumes Technology Transfer Platform (GL-TTP).

Source: AEP.

for health benefits. The conclusions of the Toledo meeting may also help us to use this new knowledge to breed plants with increased contents of beneficial ANFs. However, we must be aware of the fact that an ANF that is beneficial to man may not always be desirable in the feedstuffs used for animal production. ■

Sources: Arpad Pusztai and Susan Bardocz, Nutritional/Biochemical Consultants for GenOk (Institute of Gene Ecology), Tromsø, Norway. (a.pusztai@freenet.co.uk; s.bardocz@connectfree.co.uk) and Mercedes Muzquiz, Dept of Food Technology, SGIT-INIA, Madrid, Spain. (muzquiz@inia.es)

## FABA BEAN 2006

### International workshop on faba bean breeding and agronomy

25–27 September 2006 (Córdoba, Spain)

#### FIRST ANNOUNCEMENT

The workshop will be a forum for assessing the 'state of the art' concerning faba bean production, for identifying research issues, to establish research priorities and to promote collaborative research among international scientists.

The following issues will be considered:

- Genetic resources
- Agronomy
- Quality
- Stress resistance
- Breeding (classical and MAS)

The workshop will be organised to facilitate exchanges between participants to:

- promote existing faba bean networks by joining skills (pathology, genetics, physiology, chemistry, agronomy);
- facilitate exchanges and sharing of methodologies and strategies between leguminous crops;
- identify priorities in faba bean breeding, management and marketing

**Day 1:** State of the art of 'Agronomy', 'Farming systems' and 'Genetic resources'

**Day 2:** State of the art of 'Desirable traits: Quality and stress resistance'

**Day 3:** State of the art of 'Breeding strategies', Results and conclusions of the workshop.

Formulate a 'White Paper' that will include a consensus of opinion of the participants on the objectives and approaches needed for an increase in faba bean production and consumption. Proposal for new collaborative international networks.

The workshop will include plenary oral presentations, short oral communications illustrating poster presentations and network meetings and will allow both formal and informal interactions. Participation of junior and senior researchers is expected and encouraged.

#### Expression of interest

We are currently trying to raise funds and apply for grants for the workshop organisation, so to make the planning easier in the next months **we need your response by the 30 November 2005 to know if you and/or your collaborators are willing to participate in such a workshop, the people from your lab who would attend, their names and emails.** This will provide an email list of all potential participants, and allow us to make the reservations for the facilities. A registration fee of €300 to cover registration, documentation and meals will be required. This will not include accommodation, but access to a range of accommodation at different rates will be facilitated.

#### Contacts:

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Diego Rubiales (ge2ruozd@uco.es)



# PhD theses

## Characterisation and mapping of quantitative trait loci related to pea yield (*Pisum sativum* L.)\*

*Charakterystyka i mapowanie loci cech ilościowych związanych z plonowaniem grochu (*Pisum sativum* L.).*

by Magdalena GAWLOWSKA\*\*

The aim of the thesis was to identify loci affecting yield-related traits. The results were compared with those published earlier for the population Wt 10245 x Wt 11238 (2). 116 lines of F<sub>2</sub> progeny and 109 F<sub>4</sub> families of a cross combination Wt 11238 x Wt 3557 were used. The map was constructed using Mapmaker/Exp. v.3.0 and JoinMap v.3.0 software. The integration of the Wt 11238 x Wt 3557 map and the Wt 10245 x Wt 11238 map was performed by JoinMap v.3.0 software. Interval mapping was used to check the presence of a putative QTL.

Eighty-six markers were attributed to 10 linkage groups, related to seven chromosomes of the pea genome and to one additional group. Identification was possible thanks to 22 reference markers (published by the *Pisum* Linkage Map Committee) and eight AFLP common markers (4). The term 'common marker' stands for the markers revealing polymorphism in Wt 10245 x Wt 11238 and Wt 11238 x Wt 3557 populations. The total length of the map was 841 cM. The average distance between markers was 14 cM.

Forty-five QTLs were identified and characterised. Eleven loci were connected with plant morphology (stem length, internodes number, internodes number to the first pod, peduncle number). The interval mapping revealed that 30 QTLs accounted for yield structure – seed yield, pod number, seed number, 1000–seed weight. Four QTLs influenced seed protein content. Among 99 loci, three common QTLs (3%) were detected in both segregating populations. An additional QTL (influencing internodes number to the first pod) was common in both populations, but data came only from one generation (F<sub>4</sub>). Common QTLs were connected with yield structure (1000–seed weight, seed yield, seed number). The localisation of some common QTLs was confirmed by some published reports (1, 3, 4).

The application of QTLs in breeding (e.g. marker-assisted selection) requires the results to be confirmed in different years and cross combinations. ■

- (1) Ellis, T. H. N. *et al.* (1993). *Pisum Genetics* **25**, 5–12.
- (2) Irzykowska, L. *et al.* (2001) *Pisum Genetics* **33**, 13–19.
- (3) Timmerman-Vaughan, G. M. *et al.* (1996). *Theor. Appl. Genet.* **93**, 431–439.
- (4) Weeden, N. F. *et al.* (1998) *Pisum Genetics* **30**, 1–4.

\*PhD thesis 2004, Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Poland.

\*\* Email: mgaw@igr.poznan.pl

## Competition and complementarity in annual intercrops – the role of plant available nutrients\*

by Mette KLINDT ANDERSEN\*\*

The greatest intercrop advantages are attained when the species that are mixed differ markedly either morphologically, phenologically or physiologically. The mixture of a nitrogen-fixing legume and a non legume is the most common intercrop combination and in Denmark this is usually a pea (*Pisum sativum*) – barley (*Hordeum vulgare*) mixture.

In two field studies and one pot study the link between crop diversity, productivity and nutrient use was evaluated. The impact of crop density and the relative frequency of crop components in pea–barley intercrops was determined and the methods traditionally used to study the effects of intercropping compared with sole cropping were evaluated.

The mixture of a nitrogen-fixing crop and a non-fixing crop outyielded comparable sole crops. As a result of the legumes' ability to use atmospheric nitrogen, which increased through competition for soil nitrogen from barley and rape, the intercrops displayed complementarity with respect to nitrogen use. Increasing the number of intercrop components from two to three did not give rise to greater yields or resource use.

The competitive dynamics at play between the component crops of an intercrop are to a large extent determined in the early growth phases. Barley was the fastest emerging crop component and thereby gained a head start on the growth of pea and rape. Growth tends to be self-compounding and early advantages often lead to advantages throughout the growth season. However, conditions relating to the growth environment or cropping strategy may alter this picture. The soil nitrogen availability and cropping density had a great impact on the relative competitive strength of the studied crops. At low soil nitrogen availability the pea crop had a great advantage and as cropping density was increased the pea crop became increasingly dominant.

Intercrop research has to a great extent used an experimental design that includes two crops grown as sole crops and in proportional mixtures that relate directly to sole crops (the proportional replacement design). It is a simple design that has been valuable to show that intercrops may be more resource use efficient and productive than comparable sole crops. However, to understand underlying competitive mechanisms it may not be the most appropriate design. Experimental designs that include different crop densities and proportional mixtures of crop components (response surface design) could be more valuable.

Competitive hierarchies between crops may change over time and collecting data from several harvests throughout a growth season allowed us to point at specific structuring factors. ■

\*PhD thesis 2005, Department of Agricultural Sciences, The Royal Veterinary and Agricultural University, Copenhagen, Denmark.

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# GERMINATE: a database designed to integrate genotypic, phenotypic and genetic resources data

*Une base de données conçue pour intégrer les données génotypiques, phénotypiques et les ressources génétiques*

by Jennifer LEE,\* David MARSHALL\*\* and Andrew FLAVELL\*

Extensive genetic resource collections have been carefully developed to catalogue the genetic diversity necessary for the success of worldwide plant breeding programmes (4, 5). Such collections typically contain thousands of plant samples per species and in some cases more than 100,000 distinct lines or accessions (2). Documentation systems are a vital part of maintaining these collections in order to preserve information about the accessions and make it easily accessible to the user. With the advent of high-throughput molecular technologies, researchers have the capability to routinely genotype thousands of accessions at a time. Furthermore, increased sophistication in the methods, such as those for characterising phenotypic descriptive information or pedigrees, have added to the burden, increasing the pressure on existing documentation systems. The ability to integrate data of differing types will determine the complexity of analysis that can be carried out on accessions and data from these collections. To meet some of the challenges faced by the genetic resources community we have developed the GERMINATE database (<http://germinate.scri.ac.uk/germinate/>) which is designed to integrate genotype and phenotype information for genetic resource collections (3).

## Accommodates a broad range of datasets

One aim of the GERMINATE database is to describe genetic and phenotypic information in a generic way so that the

storage of data is flexible enough to be used with a broad range of data types and with any plant species. Databases are often designed around a specific genetic system, such as inbreeding diploids, or a specific molecular technology, such as microsatellites. Rapid advances in genotyping technologies necessitate corresponding modifications to such databases. Our generic approach avoids many of these problems and accommodates datasets ranging from simple to complex in the same database framework. We have also aimed to provide a platform that can be used to implement emerging data standards such as structured formats for describing experiments.

PostgreSQL (<http://www.postgresql.org/>) was selected for the development of the database because it meets all the criteria we required for development of an open source, scalable database. Implementing GERMINATE in PostgreSQL means users can run GERMINATE on a desktop computer (including PCs running Windows or Linux and Apple Macs running OSX) for small local databases, while it can also be scaled up to run large public databases on a server (including Linux and Unix machines).

## Controlling data integrity

Any database project must consider data integrity and curation issues. PostgreSQL fully implements the features that help to maintain referential integrity within the database, and these prevent invalid or inconsistent data being entered or maintained in a database. We can use these features to check the validity of data as it is entered into the database. For data entries that are restricted to a set of values or a controlled vocabulary we can implement a check on data as it is entered to ensure that it falls within the required values before

it is inserted in the database. Additionally, we use PostgreSQL functions to determine if data being entered are already present in the database. Such functions can be iterated through a dataset and only information that is not already present in the database will be added. However, there are many cases where data cannot be checked automatically by the database, such as spelling errors or deciding if two accessions are in fact duplicated and should be merged. Such data must then be curated. We are in the process of developing a user-friendly, web-based loading interface which will allow users to enter data and have it checked against existing data which are already present in the database. In addition, we plan to deploy a web-based curation tool that will dictate how much control a user has over data in the database and which data they have the right to modify.

Initial development of the GERMINATE database focused on genotype, phenotype and passport data. However, the underlying structure can potentially be used for a much broader range of data. The GERMINATE database has been tested for pea, wheat, barley, brassica, and lettuce data, with various genetic marker data types, genetic mapping data, trait data, and passport data. We have tested a range of datasets in GERMINATE; genetic marker datasets composed of between 10 and 3,000 accessions and evaluated with 10 to 100 markers, trait datasets comprised of 50 to over 2,000 accessions and passport datasets including more than 10,000 accessions have all been tested in GERMINATE. More complex datasets, such as those with quality information for each data point, additional information about markers (such as primer sequences and PCR conditions), and linking marker information to genetic map locations have

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also been loaded in GERMINATE. In all cases GERMINATE has performed at a level equal to or greater than that required.

## A range of interfaces

There is a range of interfaces which may be used to interact with a GERMINATE database. A web-based Perl-CGI interface has been developed within the GERMINATE project and can be used to interact with public databases on the germinate server (<http://germinate.scri.ac.uk/germinate/interface.html>). This is a light-weight interface that can be used for simple searching and browsing of the public GERMINATE databases. The Genomic Diversity and Phenotype Connection (GDPC) ((1), <http://www.maizegenetics.net/gdpc/>) can also be used as an interface to GERMINATE databases. GDPC uses a set of described objects to retrieve data from databases, an approach that allows users to integrate data from two or more disparate data sources in a single query. GDPC can be connected directly to a local database and/or remote databases using a popular computer technology called web services. Web services are well-defined objects that can be published over a network and can be accessed easily through a computer system firewall therefore making them broadly utilisable. Both the Perl-CGI and GDPC browser options can be used with no bioinformatics experience. Examples of the GDPC and Perl-CGI browser interfaces are shown in Figures 1 and 2, and tutorials on getting started with using these interfaces can be found at <http://germinate.scri.ac.uk/germinate/tutorial.html>. Alternatively, users experienced in SQL may interact with the database at the SQL level to form any complexity of queries desired.

## Analysis and visualisation tools

In addition to the web-based loading and curation tools which are being designed we intend to maximise the usefulness of data stored in GERMINATE databases by developing and identifying a range of analysis and visualisation tools which will meet user needs. Two tools have already been made available through GDPC: the GDPC browser (<http://www.maizegenetics.net/gdpc/>) and TASSEL (<http://maizegenetics.net/bioinformatics/tasselindex.htm>). Currently under development is a Graphical Genotype

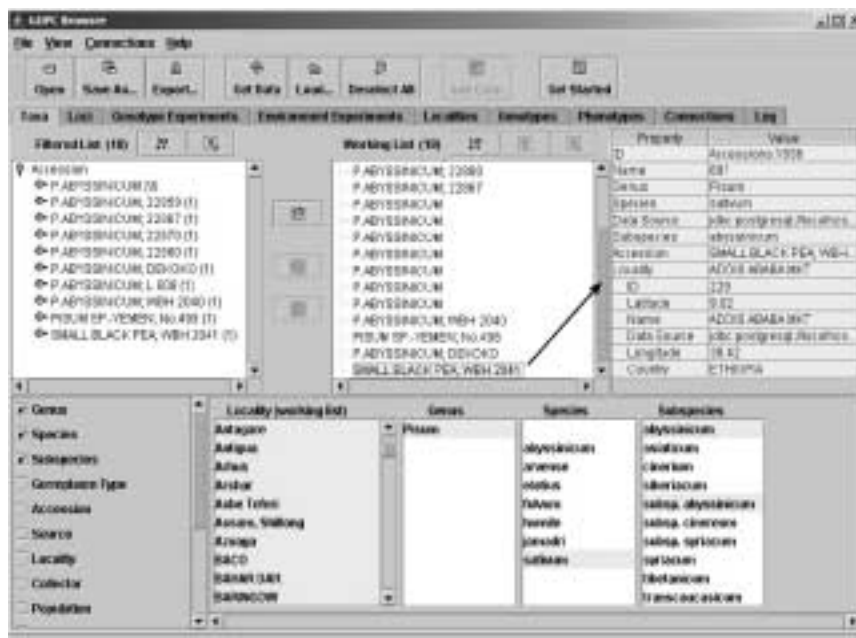


Figure 1. Example of the GDPC Browser interface to GERMINATE. The queries shown retrieved *Pisum sativum* subspecies *abyssinicum* accessions and returned taxonomy and locality information.

accession	accession ID	genus	species	subsp.	accession name	origin	latitude	longitude	Accession Count
GDRC011	1206	Pisum	sativum	abyssinicum	P.ABYSSINICIUM	ETH	8.35	35.16	3
GDRC011	126	Pisum	sativum	abyssinicum	P.ABYSSINICIUM	ETH	8.22	35.42	3
GDRC011	127	Pisum	sativum	abyssinicum	P.ABYSSINICIUM	ETH	8.42	35.42	3
GDRC011	161	Pisum	sativum	abyssinicum	SMALL BLACK PEA	ETH	8.62	35.42	3
GDRC011	1837	Pisum	sativum	abyssinicum	P.ABYSSINICIUM	ETH	11.33	36.6	3
GDRC011	1862	Pisum	sativum	abyssinicum	P.ABYSSINICIUM	ETH	11.33	36.6	3
GDRC011	1937	Pisum	sativum	abyssinicum	P.ABYSSINICIUM	ETH	12.3	35.31	3
GDRC011	1966	Pisum	sativum	abyssinicum	P.ABYSSINICIUM	ETH	12.46	34.31	3
GDRC011	3	Pisum	sativum	abyssinicum	P.ABYSSINICIUM	ETH	12.46	34.31	3
GDRC011	1878	Pisum	sativum	abyssinicum	P.ABYSSINICIUM	ETH	13.2	35.28	3
GDRC011	2046	Pisum	sativum	abyssinicum	P.ABYSSINICIUM	ETH	13.29	35.28	3
GDRC011	2308	Pisum	sativum	abyssinicum	P.ABYSSINICIUM	ETH	13.29	35.28	3
GDRC011	2309	Pisum	sativum	abyssinicum	P.ABYSSINICIUM	ETH	13.29	35.28	3
GDRC011	130	Pisum	sativum	abyssinicum	P.ABYSSINICIUM	ETH			3
GDRC011	1457	Pisum	sativum	abyssinicum	P.ABYSSINICIUM	ETH			3
GDRC011	1458	Pisum	sativum	abyssinicum	P.ABYSSINICIUM	ETH			3
GDRC011	1558	Pisum	sativum	abyssinicum	P.ABYSSINICIUM	ETH			3

Figure 2. Example of the Perl-CGI web interface to GERMINATE. The queries shown retrieved *Pisum sativum* subspecies *abyssinicum* accessions and returned taxonomy and locality information.

Tool that will display graphically the distribution of alleles at loci across taxa, viewed by genetic linkage map position. Programs such as DIVA for displaying geographical information and statistical analysis modules written in programming languages such as R are also being considered for use with GERMINATE. As user needs are defined further we will identify existing tools which can be connected to GERMINATE and develop new tools which will be of use to researchers. Up-to-date information on all these matters will be posted at the GERMINATE website (<http://germinate.scri.ac.uk/germinate>). ■

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## The physiology of the pea crop

### Agrophysiologie du pois protéagineux

March 2005, French, 281 pages

Nathalie Munier-Jolain,  
Véronique Biarnès, Isabelle

Chaillet, Jérémie Lecoeur and  
Marie-Hélène Jeuffroy (Eds)

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2-86492-679-2

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du Végétal and Agro Montpellier.

This book was written by researchers from different disciplines and agricultural development specialists working together for many years on the production of plants rich in protein in France and in Europe. It reviews the current knowledge on the physiology of the pea crop.

This book, in three parts, presents first the vegetative and reproductive development, the growth under non-limiting conditions and the nitrogen nutrition of the pea crop. Secondly, the effects of abiotic and biotic stresses on the development, the growth and the nitrogen uptake by the plant are studied. Finally, a global model of the functioning of the pea crop is proposed as a tool for the diagnosis of yield-limiting factors.

This review with 10 chapters and 367 bibliographic references, is intended for researchers, academics, breeders, and extension specialists. It is relevant to all investigations of the physiological basis of the functioning of a pea crop.

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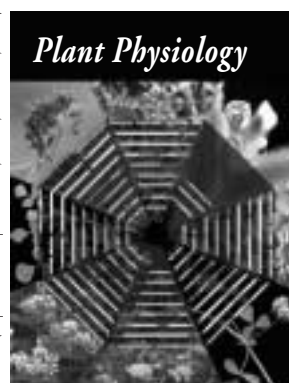
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## Plant Physiology

April 2005 Volume 137,  
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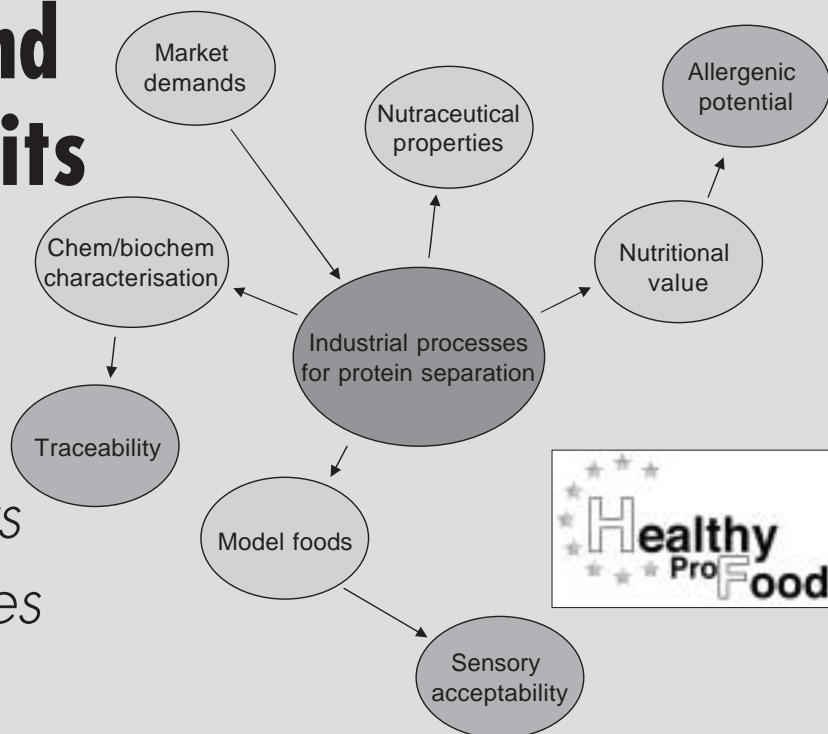
Following an Editorial, 'Translational' legume biology. Models to crop, by Garry Stacey and Kate Vandenbosch, there follow updates on

sequencing *Medicago truncatula* and *Lotus japonicus*; nematodes: sophisticated parasites of legumes; comparative genomics of legumes; protein degradation controls nodule development; and legumes symbiosis. Further papers are on genome analysis (1), biochemical processes and macromolecular structures (2), development and hormone action (3), environmental stress and adaption (1), genetics, genomics and molecular evolution (3), plants interacting with other organisms (9), and whole plant ecophysiology (2).

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# Food uses and health benefits of lupins

*Lupin: atouts santé et produits agro-alimentaires*



Overview of the Healthy-Profood project.

**L**upin seeds have a high protein content and have been consumed for centuries especially in Mediterranean countries. However, they are still under-exploited in the food industry.

Partners in Healthy-Profood<sup>1</sup> have carried out scientific and technological work on lupin seeds for the production of food ingredients useful in different food applications, and they have investigated their role in the prevention of cardiovascular diseases.

This special report starts with an overview of the project and its achievements. It also reports the findings of a survey carried out in 2004 to assess the perception of European industrialists and retailers regarding lupin in the market of plant-based ingredients and foods.

The extracts from lupin seeds vary according to the method of processing employed. The production of three different types of lupin ingredients is described here, together with some of their food applications.

The risk of lupin allergy is explored and methods of tracing lupin in food ingredients and end-products are described.

The potential role of lupin protein in atherosclerosis prevention is an additional asset and the most recent progress in this field is described. ■

<sup>1</sup>HEALTHY-PROFOOD is a project financed by the EU, from 1 January 2003 to 31 December 2005, within the 5th Framework Programme (QLRT 2001-002235). Full title: Optimised processes for preparing healthy and added value food ingredients from lupin kernels. Coordinator: Dr Anna Arnoldi (DISMA, Italy) (<http://users.unimi.it/healthyp/index.html>)

Les graines de lupins, riches en protéines, sont connues et consommées depuis des siècles, tout spécialement dans le pourtour Méditerranéen. Cependant elles sont toujours peu valorisées en agro-alimentaire de nos jours.

Les partenaires du projet Healthy-Profood<sup>1</sup> ont conduits des travaux scientifiques et technologiques sur l'utilisation des graines de lupins pour la production d'ingrédients agro-alimentaires utiles pour différents types de préparations et ils ont étudié leur rôle dans la prévention des maladies cardio-vasculaires.

Un aperçu général du projet et de ses conclusions ouvre notre dossier spécial. Sont ensuite rapportés les résultats d'une enquête menée en 2004 qui a permis de mieux connaître la perception des industriels et distributeurs européens sur le potentiel du lupin dans le marché des ingrédients et produits d'origine végétale.

Les produits d'extraction des graines dépendent des variantes technologiques. Sont présentés ici la préparation de trois types d'ingrédients de lupin et plusieurs exemples d'applications pour préparer des produits finis.

Le risque d'allergénicité du lupin est également abordé et les méthodes de traçabilité du lupin au sein des ingrédients et produits finis sont explorées.

Le rôle potentiel de la protéine de lupin pour la prévention de l'athérosclérose est un atout supplémentaire : le dernier article résume les avancées récentes sur ce sujet. ■

<sup>1</sup>HEALTHY-PROFOOD est un projet financé par l'UE (QLRT 2001-002235) du 1er janvier 2003 au 31 décembre 2005, dans le cadre du 5<sup>e</sup> Programme Cadre de Recherche et Développement Technologique, Son titre: "Optimisation des procédés technologiques de préparation d'ingrédients alimentaires, à haute valeur ajoutée et apportant un bénéfice santé, à partir des amandes de lupin". Coordinatrice: Dr Anna Arnoldi (DISMA, Italie) (<http://users.unimi.it/healthyp/index.html>)

# The Healthy-Profood project: overview and main outputs

*Le projet Healthy-Profood et ses conclusions*

by Anna ARNOLDI\*

**H**ealthy-Profood (QIK1-2002-02235), financed by the European Commission within the 5th Research Framework Programme, is a three-year research project, bringing together six research centres, four companies, four consumer associations and AEP (see *Grain Legumes* No. 39, 18–19).

This project was planned in a climate of uncertainty, with the European market for food ingredients facing increasing challenges, in particular cost effectiveness, excellent functionality, demand for nutritionally added-value products, demand for healthy and nutraceutical properties. In this context, lupin seemed a very good candidate to become a useful source of food ingredients, since the protein content is high (35%–40%), and the level of antinutritional components low.

The main objectives of the Healthy-Profood project are the following:

- To optimise economically competitive and environmentally sustainable processes for the preparation of food ingredients with optimal technological, sensory, and nutritional characteristics, based on lupin kernels;
- To assess the potential health benefits of these food ingredients in hypercholesterolaemia, hypertension, and diabetes;
- To develop protocols for the preparation of ready-to-serve food products based on lupin food ingredients and to determine the consumer acceptance;
- To promote lupin-based ingredients and food items by active participation of European consumer associations.

## Ingredients separation optimised

The project has permitted the development of two reproducible industrial

processes for the separation of protein concentrates and isolates from white lupin and yellow lupin kernels. The process parameters were optimised in order to improve their sensory, chemical, and techno-functional features. The latter were selected for their relevance in the formulation of end-products: foaming activity and stability, interfacial properties, emulsifying properties, gelling properties, water/fat binding capacity, and convenient shelf-life. The thermal damage, measured as furosine and HMF, is lower or comparable to other commercial food ingredients, both of animal or vegetable origin and lysine unavailability is negligible. Additionally the food ingredients were also characterised accurately for the presence of vitamins and minor antinutritional compounds, such as alpha-galactosides, alkaloids, isoflavones, and saponins. Proteomic techniques enabled the composition of the industrial lupin protein isolates and concentrates to be assessed, and demonstrated that the most important protein fractions maintain their integrity reasonably well during processing.

The potential allergenicity of lupin proteins has been investigated, with particular reference to the possible cross-reactivity with other legume proteins (soyabean and peanut). Weak cross-reactivity was observed, but only in the case of the minor protein component gamma conglutin. Methods to assure the traceability of this protein fraction are under investigation. An ELISA immuno-assay has been developed, which can detect this protein efficiently in unprocessed products, and a PCR method is under evaluation for application in processed foods.

## Palatable model foods

A large range of model foods was prepared using lupin flour or lupin protein



isolates. The food items were selected taking into account the requirements of the EU food industry for protein ingredients, as well as consumer trends for their sensory and nutritional characteristics, and industrialists' specifications regarding novel food items. The main items were lupin beverages (prepared with different recipes and flavours), some snacks, muffins, spaghetti, biscuits (prepared with different recipes and flavours). These items were submitted to a sensory evaluation by domestic panels and also by a panel of consumers (in collaboration with a consumer association) and, with only a few exceptions, were found to be very palatable. Amounts of lupin protein ranging between 5% and 10% are generally well accepted in most food applications.

## Health benefits

Considerable effort was dedicated to the investigation of the biological properties and health benefits of lupin proteins. Studies on animal models (rats and rabbits) confirmed that lupin proteins have a significant hypocholesterolaemic potential. In a clinical study with healthy volunteers who consumed a lupin beverage instead of milk for three months, nobody showed any adverse reaction and the acceptance and compliance were good. Most subjects presented a reduction of total and LDL-cholesterol levels and of homocysteine levels. An unexpected significant decrease in blood pressure was also observed, while oxidative parameters were unchanged.

AEP and the consumer associations have been very active in disseminating the results of the project among end-users and consumers and the final conference will take place in Milan, on 9–10 November 2005). ■

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# The perception of lupin in the European food industry

## *La perception du lupin par l'industrie agro-alimentaire européenne*

by Anne SCHNEIDER\*, Katell CRÉPON and Evelyne FÉNART

In 2004, in the context of the HEALTHY PROFOOD research project<sup>1</sup>, AEP carried out a study of the market for ingredients made from vegetable protein for use in the European food industry. The 'Business to business' study aimed to determine the place of lupin in this market, and the perception of industrialists (ingredient producers or users) and retailers regarding lupins and lupin-based ingredients.

Following studies of the literature and informal interviews, 25 formal interviews with industrialists using detailed questionnaires were carried out in different European regions including France, Germany, UK, Italy, the Netherlands and Finland. The survey results can be requested at AEP headquarters and are developed in (1). Figure 1 summarises the main features of the lupin market.

Market analysis shows that there are two types of products:

- (i) 'lupinfoods' vegetable milk type ('lupin milk') and vegetable milk-based foods (i.e. 'lupintofu'), 100% lupin-based products either sold directly to the consumer or used by the food industry to formulate food products (drinks, vegetable sausages, vegetable 'pâtés', etc.)
- (ii) lupin-based vegetable ingredients which are 'Rich protein ingredients (not additives) processed from oilseeds, grain legumes or cereals: soya, pea, lupin, faba bean, wheat, containing at least 40% proteins/DM' (Codex Alimentarius).

### Two possible market strategies

First there is the substitution market, where lupin protein replaces soya proteins to avoid the GMO issue<sup>2</sup>. Currently this market is

increasing, but it remains irregular and fragile, because the GMO issue is not yet clear in European countries. Second, there is the lupin-specific market, where its non-GMO, healthy image and yellow colour distinguish it from the soya protein and make it more efficient in some sectors, such as the bakery sector and for the organic market.

### Bulk market or niche organic market

In 2004 there were three established providers of lupin vegetable proteins in the EU market. They use different lupin species and this makes the raw material characterisation difficult. One producer is part of a cooperative group that includes growers who ensure a regular supply of seeds to produce lupin-derived products within the group, and to supply its food industry clients.

Ingredients producers can have a proactive marketing strategy proposing formulation and products for the food industry. In other situations, vegetable protein

distributors may propose alternative protein ingredients to replace soya or, occasionally, they receive specific requests from their clients. Conventional ingredients represent a bulk market but its development depends upon the strategies of different companies.

Food retailers have an increasingly significant role because they can exaggerate the views of consumers and can exploit the trends to their own advantage.

Lupinfoods are usually linked to the organic market, which is a niche market with a specific trademark system and retailers. The organic market and lupinfoods are in close contact with consumers and have a major role in promoting lupins.

### More information is needed

It is clear that the lupin price is a key bottleneck for the lupin market. Significant quantities and regular supplies are required in order to characterise lupin seed fully. Competition with soya products is also a major constraint, and either a lower lupin price, or the promotion of lupin-specific

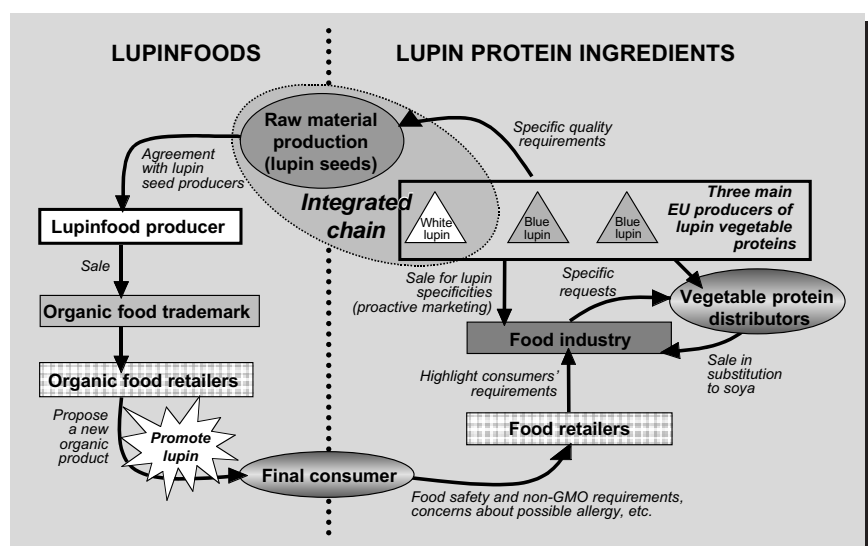


Figure 1. Schematic structure of the lupin market (issued from Healthy-Profood survey 2004).

Continued overleaf...

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... continued from previous page

attributes is required. To date there has not been a synergy of effort to further lupin-based product development.

There is a general lack of information for consumers about the nutritional benefits of lupin, but potential industrial users also have poor knowledge of new and improved crop varieties, lupin seed composition and the possible uses of lupin seeds and ingredients. In contrast, the specific characteristics of lupin are fully appreciated when lupin is tested or used at the industrial R&D level, and there is clear interest in lupin foods for the organic market.

Most of the disadvantages associated with lupin are the classical reaction towards a new source of protein ingredient on the market. It is clear that a marketing strategy targeting industry will be facilitated when technical data and pre-competitive evidence regarding the interest in lupin ingredients are available. This could convince both industrialists and retailers to invest in lupin-based products.

Additional private investment is needed to disseminate basic information to the general public about plant protein sources and the potential of lupin for food and health. This would also provide retailers, who are powerful players in the supply chain, with more arguments to promote lupin-based end-products. ■

(1) Schneider, A. *et al.* (2005). Proceedings of the final conference of Healthy-Profood, Milan 9–10 November 2005.

<sup>1</sup>Healthy-Profood (Optimised processes for preparing healthy and added-value food ingredients from lupin kernels) is a multinational project supported by 5th RDT Framework Programme of the European Union (QLRT 2001-002235) from 1 January 2003 to 31 December 2005, and coordinated by Dr A. Arnoldi (DISMA, Italy).

<sup>2</sup>Concern about the safety of genetically-modified organisms.

## Lupin food ingredients and lupin-based food products

### *Ingrédients alimentaires à base de lupins et produits transformés*

by Udo KNAUF\*, Alice SEGER\*\*, Christian BAGGER\*\*\* and Jürgen BEZ\*

The success of new foods is mainly build on positive sensory appearance (colour, smell, taste, texture in the mouth) and consumer acceptance. This in turn depends on the ingredients used and on the processing methods. The ingredient's sensory properties must be compatible with the food and the techno-functional properties need to be sufficient to build up the required texture. Along with low production costs and a positive consumer acceptance the ingredient shows the potential for success. The final composition and the processing method of the food must take into account the single properties and interaction potential of the ingredients. Lupin ingredients show high potential for successful new and healthy food applications.

#### Different types of ingredients

The properties of food ingredients like form, colour, taste and functionality are strongly dependent on their origin and their processing. In this project *Lupinus albus* and *Lupinus luteus* were used to produce food ingredients. These species – and to a lesser extent their cultivars – differ in fat content, protein content, protein solubility and protein functionality. To produce lupin food ingredients various process steps like de-hulling, grinding, extraction, precipitation, filtration, modifications and drying as well as their respective process conditions like temperature, pH values, cut-offs etc. can be applied. With this wide range of processing options different types

of ingredients like flour, meal, grits, concentrates, isolates and hydrolysates can be produced. The processing methods for three different ingredients are described briefly.

#### Lupin flour and grits

Lupin flour and grits offered by Terrena (LUP'INGREDIENTS range) are produced from sweet *Lupinus albus*. The seeds are cleaned, then toasted and de-hulled. The de-hulled seeds are ground or crushed and sifted in order to obtain lupin flour and grits. Compared with raw soy ingredients, lupin ingredients are significantly higher in protein, contain three times the fibre and half the lipids, and have much lower energy content (Table 1). Also lupin ingredients are high in micronutrients (including group B vitamins and minerals: magnesium, iron, phosphorus, copper and calcium). Lupin flour and grits offer the same nutritional values and complementary food application. Lupin flours bring functional properties to dietetic products, bread, biscuits, gluten free products etc: it is a natural emulsifier and colouring agent, a taste enhancer and offers a high water-binding capacity for clean labelling products and economical formulations. Lupin grits bring nutritional value, an original colour, a hazelnut-like taste and crispness to special bread or biscuits.

#### Lupin concentrates/isolates

The BIORAF Denmark process for preparing Protein VI is based on water as the extracting agent and starts with de-hulled *Lupinus luteus* flour (Figure 1). The lupin flour is prepared by roller milling, sieving and air sifting. The flour is extracted gently with water in weakly alkaline conditions and the solution is separated into a solid

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and an aqueous phase by decanter type centrifugation. The solid phase, which contains the fibre fraction, can ideally be submitted to a washing step before drying. Potentially, the fibres can be applied in food products. The aqueous phase, which contains the water soluble components such as the storage proteins, is separated into high and low molecular weight fractions by ultra-filtration. The low molecular weight fraction, which contains the low molecular weight components such as sugars, can be evaporated and used as feed. The high molecular weight fraction, which contains 85–90% protein (N\*6.25) and 5–10% lipids, is spray dried. The spray dried protein product called Protein VI exhibits excellent emulsifying properties.

### Lupin isolates type E and type F

The Fraunhofer IVV process (Figure 1) starts with white flakes from *Lupinus albus*. These are de-hulled and flaked lupin seeds that are de-oiled by hexane and gently de-solventised. The white flakes undergo an acid pre-extraction step at pH 4.5 to remove soluble sugars and alkaloids. A decanter centrifuge separates the solids that are extracted in neutral conditions (pH 6.8–7.2) to dissolve the storage proteins. After removing the fibres by another decanter centrifuge, the protein solution will be precipitated by pH-shift to the iso-electric point (pH 4.5). The precipitated proteins

are separated as curd by a separator centrifuge. The curd is neutralised and spray dried. This protein isolate contains more than 95% protein and is called type E because of its excellent emulsifying properties. The supernatants of the pre-extraction and of the precipitation are filtered jointly to separate the proteins from low molecular weight substances like sugars, alkaloids and salt. After this ultra- and dia-filtration (cut-off 15 kDa) the protein solution is spray dried. This protein isolate contains 85–90% protein and is called type F due to its excellent foaming properties. Both isolates have less than 1.5 % of fat and neutral sensory properties.

### Several food applications

With respect to the sensory properties and the techno-functional properties like emulsifying, foaming and gelling, suitable food applications have been selected. Different sectors like bakery goods (muffins, biscuits), emulsified foods (drinks, imitation cream, dressings) and extruded foods (pasta, snacks) have been developed successfully. Two examples are described briefly.

### Lupin drink

The lupin drink is a ‘lupin-milk’ made simply from lupin flour and water. The drink is produced with the following steps: lupin flour and water are mixed together at an adjusted pH for best lupin protein solubility. Then insoluble fibres are separated from the soluble proteins by settling. The supernatant containing the soluble lupin proteins is ultra-filtered in order to eliminate small molecules such as sugars. Finally the drink is sterilised by UHT (ultra high temperature) to increase the shelf-life but without impairing the taste, colour or nutritional value. The result is a white base-drink with a slight touch of yellow and a nice hazelnut-like taste. This basis for new drinks is a product of high-quality and nutritional value: according to the French regulation, the lupin drink is a ‘source of omega 3, a ‘source

**Table 1. Nutritional values (per 100 g) of lupin flour and grits compared with soy flour.**

	Lupin flour and grits		Soy flour	
Protein	41.0 g		34.5 g	
Carbohydrates (no fibre)	10.5 g		25.6 g	
Fibre	31.0 g		9.6 g	
Lipids	10.2 g		20.6 g	
SFA	% of TFA	1.5 g 14.2%	2.3 g 11.2%	
MUFA	% of TFA	6.0 g 59.2%	4.6 g 22.3%	
PUFA	% of TFA	2.7 g 26.6%	11.7 g 56.8%	
Omega 6	1.4 g		10.3 g	
Omega 3	0.8 g		1.4 g	
Ratio Omega 6/Omega 3	1.8		7.4	
Energy value	297.8 kcal		425.8 kcal	

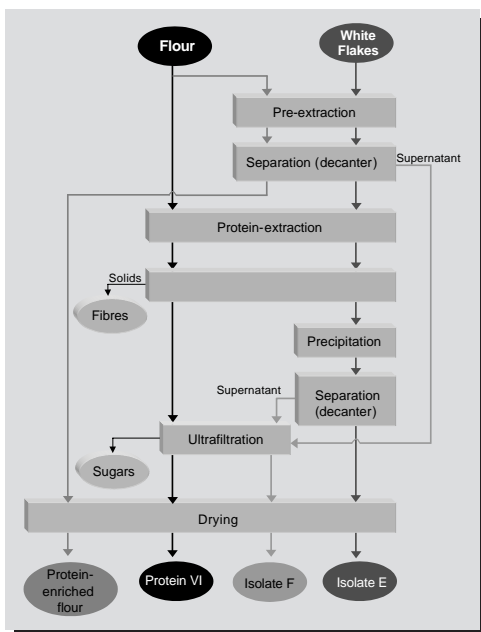
SFA = saturated fatty acids; MUFA = mono unsaturated fatty acids; PUFA = poly unsaturated fatty acids, TFA = total fatty acids.

of calcium and iron’ and ‘rich in magnesium’. From this base drink it is now possible to produce highly nutritional vegetable drinks with different flavours and also other products such as ‘vegetable yoghurt’ and ‘vegetable cream’.

### Muffins

Muffins were produced using lupin protein isolate type E in concentrations of 1.5–3.7% (w/w) as a substitute for egg and milk protein. The best texture and taste was achieved at 2.2%, replacing 100% of the milk and egg of the original recipe. The rest of the composition remained nearly unchanged. This way a 100% plant-based muffin could be produced since the other ingredients, like wheat flour, sugar, sunflower oil, vanilla sugar, baking powder, citric acid, salt, lemon peel and baking soda, are also plant based. In a sensory analysis performed with a qualified panel, the lupin muffins were compared with an egg/milk-based muffin. No significant differences were reported by the evaluators, in particular regarding texture and aroma. Since the baking behaviour of the muffins did not differ significantly, the modified recipe can be made easily with the usual equipment.

To conclude, lupin offers a wide range of possible food ingredients as well as tasty and health-promoting foods which exactly meet consumer demand for innovative food products. Lupin ingredients are likely to be added increasingly in food to enhance the sensory and nutritional profile and to substitute animal-based proteins. This way lupin has the potential to reach sales levels similar to those of soy eventually. ■



**Figure 1. Production procedures for different ingredients.**



# What about the possible allergenicity of lupin?

## *Qu'en est-il de l'allergénicité éventuelle du lupin?*

by Hanne FRØKJÆR\*

**A**s lupin has only recently been introduced as a food or food ingredient on the European market, the knowledge of the allergenicity of lupin is rather limited. France was the first country that permitted lupin flour as a food ingredient in 1997, and subsequently the rest of Europe has followed. However, one fact is indisputable: ingestion of lupin flour as a constituent of a meal may cause severe allergic reactions in people at particular risk – in the worst case, anaphylactic shock. In 2002, lupin was the fourth most frequent cause of food-associated anaphylaxis in France and, in the UK, cases of anaphylactic reactions are also emerging (2, 5). Therefore, assessment of the risk of allergenicity of lupin is a necessity. A new directive on food labelling in Europe came into force in the autumn of 2004 (2003/789/EC), requiring specific listing of potentially allergic ingredients on food products. This list includes the legumes, peanuts (groundnuts, *Arachis hypogaea*) and soybeans, but not lupin or other legumes previously demonstrated to cause severe allergic reactions in soy- or peanut-allergic individuals, as a consequence of cross-reaction between the proteins.

Such risk assessment includes two questions: can lupin cause allergic reactions in patients already known to be allergic to other legumes and, can lupin by itself evoke allergy as seen by peanut and soy?

### **Patients known to be allergic**

Lupin belongs to the same family of vegetables as other potentially allergic foods, especially soy and peanut, but also pea and chickpea. Cross-reactivity between the proteins from these plants exists, and accordingly allergic reactions towards lupin proteins in individuals already allergic to,

for example, peanut or soy are to be expected. In fact, several examples of lupin allergy have been reported in patients known to be allergic to other legumes, in particular peanut, soy or pea (4, 1). This is a serious problem as several cases of life-threatening anaphylaxis have been reported after ingestion of food containing lupin flour.

### **Can lupin alone evoke allergy?**

Whether lupin as a constituent of food by itself has the capacity to induce allergic reactions remains to be established. This will only be possible either by waiting some more years and then seeing whether the frequency of lupin allergy increases, or by using animal models of food allergy. The first possibility is hampered by the widespread use of peanuts and soy in our diet. With animal models it would be possible to exclude prior exposure to other legumes, but there are no consensual models at present.

There is, however, evidence that lupin allergy due to inhalation of lupin flour exists. Both in Spain and Australia reports point at an increased frequency of lupin allergy among workers in the lupin processing industry. Accordingly, lupin may, like other plant food allergies, be most frequent in areas where lupin is grown and processed.

### **Processing to reduce the risk of lupin allergy?**

All research aimed at eliminating the allergenicity of allergic food proteins by processing has shown that it is not possible in an economically feasible way to eliminate the allergenicity of allergenic food to such an extent that no one at risk for allergic reactions to the allergen will react in a life-threatening or at least unpleasant way.

On the other hand, considering the potential beneficial effects of specific lupin proteins on health, it may be worth considering the possibility of processing to reduce the risk of inducing lupin allergy in individuals that are not already sensitised to other legume allergens. By using the most promising animal model for the assessment of food allergenicity (3), we have, within the activities of Healthy Profood, assessed the potential of lupin flour, and two industrially produced lupin protein isolates comprising different types of the storage proteins, conglutins (6). Our results demonstrated that with oral administration lupin flour had a much stronger potential than the two lupin protein isolates to induce IgE antibodies against specific lupin proteins, and that the IgE antibodies in mice reacted towards the same proteins as those demonstrated in the sera from humans allergic to lupins (4, 1). Therefore, although lupin flour apparently may cause the induction of allergy, there is reason to believe that it will be possible to produce health-promoting lupin protein isolates, which will not pose any risk for consumers that are not already allergic to lupin or other legumes, such as peanut and soy. ■

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# Tracing lupin in food ingredients and end-products

## *Outils de traçabilité du lupin dans les ingrédients et produits alimentaires*

by Alessio SCARAFONI, Elena SIRONI and Marcello DURANTI\*

Modern food safety criteria require very accurate food control. The Regulation (EC) No 178/2002 (1) defines traceability as 'the ability to trace and follow a food, feed, food-producing animal or substances intended to be, or expected to be incorporated into a food or feed, through all stages of production and distribution'. Thus, traceability of components in the food chain is an essential step for food classification and 'typification', ingredient standardisation and, most important, safety purposes. This need is especially acute nowadays, since food composition and quality are affected by a number of processing events. Traceability meets two main requirements, first, the need for producers to standardise raw materials in order to guarantee a qualitative and quantitative constancy of the end-product and, second, safety reasons for the detection of components potentially detrimental for human health. Therefore, traceability is relevant for various categories from producers through food controlling boards to consumers.

### Tracing methodologies even for complex systems

Typically, the possibility to trace a food or an ingredient is based on the peculiar properties of one or a few molecules, which are clearly and unambiguously distinguishable from the several other food components. Nucleic acids and proteins are the two classes of macromolecules that fulfil this requirement. Indeed, thanks to their specific nucleotide and amino acid sequences, that are encoded for by the

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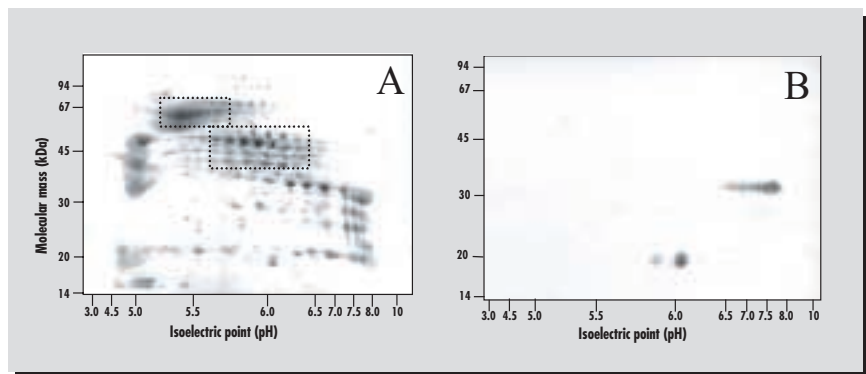


Figure 1. 2D electrophoretic map of total lupin proteins (A) and the same map reacted with anti conglutin  $\gamma$  antibodies (adapted from (2)).

genetic material of each cell type and are virtually unique for each of them, nucleic acids and proteins are the target of any tracing analytical approach. With progress in biochemical and molecular biology techniques, several methodologies are now available to detect and characterise these molecules even in complex systems.

Among the protein-based methods, bi-dimensional (2D) electrophoretic techniques are becoming widely used in the food area. This technique provides a 'picture' (map) of the protein pattern (proteome) of any biological specimen, because of its capacity to resolve complex protein mixtures (more than a thousand proteins simultaneously). Therefore, proteomics is especially suitable for tracing purposes. This technique takes account of the protein modifications, such as limited proteolysis, which may take place during food preparation and storage. The 2D maps of a given sample can be compared with standard maps prepared with fully characterised reference proteins. Moreover, protein spots can be identified individually by immuno-revelation, mass spectrometry and amino acid sequencing.

Immuno-assay technologies are based on the interaction between antibodies and antigens. They are ideal for the qualitative/quantitative detection of proteins in complex matrices when the target analyte is known. Both monoclonal and polyclonal antibodies can be used. Western and dot blots and ELISA are the three most applied methods for routine testing. DNA detection methods rely on the complementarity of two strands of DNA double helix that hybridise in a sequence-specific manner. Although several techniques are available, PCR (Polymerase Chain Reaction)-based analyses are the most commonly used. PCR methods allow the selective amplification of specific DNA regions (known as 'target') in a complex mixture of other DNA sequences. Real-time (RT)-PCR is able to quantify specific DNA sequences present in a given sample.

The use of all these approaches can be affected greatly by matrix effects. Indeed, foods are complex and dynamic systems in which several molecules can interact with proteins and DNA altering their real capacity to be detected and food processing and storage can cause analyte modification and degradation.

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### Lupin flours and proteins must be traceable

Lupins and soybeans have the highest protein levels among leguminous seeds. Furthermore, in lupin seed the content of some antinutritional compounds, including lectins, hydrolase inhibitors, saponins and antimetabolites is much lower than in soybeans and other grain legumes. Although lupin seeds have been consumed as food for centuries, especially in Mediterranean countries, lupin is still an underexploited legume seed. However, with a view to more extensive use of lupin flour and its proteins, as food ingredients in the future, it is now necessary to set up and optimise methods aimed at tracing their presence in food formulations.

In our lab several different experimental approaches are currently being investigated and tested to trace lupin ingredients in model and complex foods. Dot blotting and ELISA analytical formats have already been set up to detect specific lupin proteins in the food chain related to potential intolerance and allergenicity responses to lupin-based food intake. In addition, 2D electrophoresis has been used to create reference maps (Figure 1) from highly purified white lupin seed protein fractions ( $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  conglutinins). These proteins are industrially prepared as isolates or concentrates and are used as functional ingredients in many food formulations. This approach, which can be combined with Western blotting analysis, allows the detection of lupin proteins in complex foods by using comparative software tools (Figure 1).

To test for the presence of lupin flours in food we are setting up a PCR-based method. The test is designed to detect the presence and also to discriminate lupin from other legume flours present in the sample. The possible degradation of DNA, which can occur in real food systems and affect the success of the amplification, is also taken into consideration by using different sets of primers. The method is likely to be applicable to real food systems by the end of the year. ■

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## White lupin protein to prevent cardiovascular disease: experimental and clinical evidence

### *La protéine de lupin blanc pour lutter contre les maladies cardiovasculaires: données expérimentales et cliniques*

by Cesare R. SIRTORI\* and Marek NARUSZEWICZ\*\*

With growing epidemics of atherosclerotic vascular disease, particularly in Eastern European countries, health authorities and citizens are more and more determined to identify the best healthy food for the prevention of arterial disease. About 30 years ago, data were presented from clinical studies in hypercholesterolaemic patients (6), indicating that total substitution of animal proteins with vegetable proteins from soy resulted in a remarkable reduction in cholesterolaemia (~20% or more). Today, such findings can be obtained using synthetic lipid-lowering agents.

### More acceptable to the general public

Practical application of diets with significant changes in protein composition, for example, soy replacing meat protein do not, of course, receive general acceptability. Thus, in the last three decades, technology has helped considerably to make soy products taste better and be more acceptable to the general public. This has resulted in a more than 100-fold increase in revenues from soy-based products for human use in the Western world. Data have also been provided indicating the significant effects of these vegetable proteins on arterial lesions in animal models (1) as well as some

additional effects, for example, reducing blood pressure and body weight (2, 3).

More recently researchers on vegetable proteins have aimed to find tradition European alternatives to soy proteins, possibly of better taste and free from phytoestrogens, components of soy products which are of dubious therapeutic benefit and potentially associated with side effects (8).

White lupin (*Lupinus albus*) is a common species in Western Europe, its availability dating back to Roman times when soldiers used the seeds as a major source of dietary proteins, quite adequate for their needs. The seed of *Lupinus albus* contains more than 45% protein, virtually free from phytoestrogens and with a fatty acid composition characterised by a relative enrichment of n-3 fatty acids.

### Lupin proteins can reduce cholesterolaemia

Initial results with rats show that lupin proteins can reduce cholesterolaemia as much as soy proteins (7) (Figure 1), and preliminary data in humans suggest that these products can be taken safely and are actually preferred to soy proteins by participants in dietary studies (5). The lack of phytoestrogens makes them more suitable for younger individuals.

These preliminary ongoing human studies (5) involved a group of 18 volunteers (five men and 13 women, mean age  $48 \pm 8$  years) with mild non-familial hypercholesterolaemia (total cholesterol

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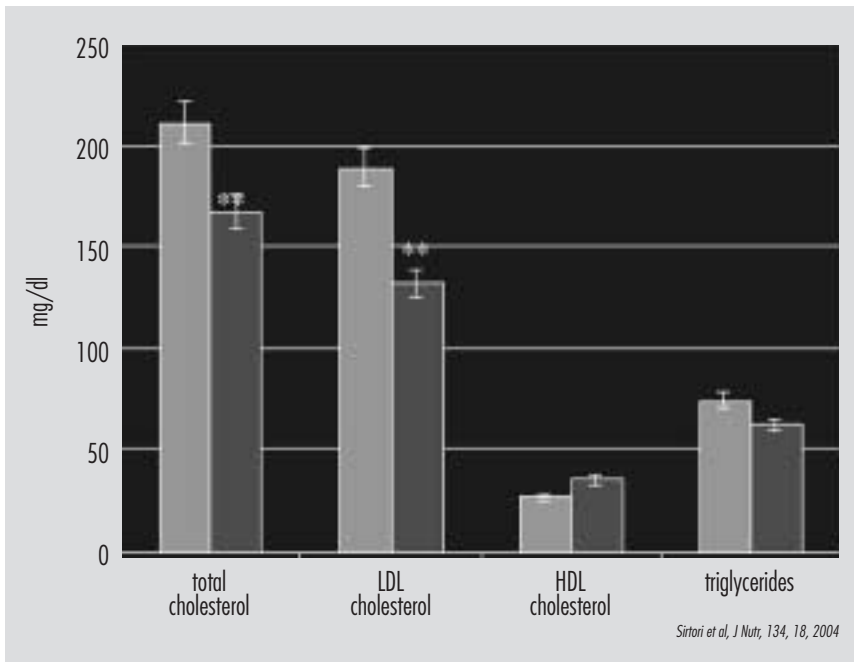


Figure 1. Lupin TPE administration to rats fed a hypercholesterolaemic diet.

246 ± 26 mg/dl). Each received a daily dose of 36.6 g lupin protein in the form of water solution (2 x 250 ml) for 30 days. During the experiment, the subjects did not change their habitual diet, and there were no side effects that would require discontinuation of lupin protein. Moreover, no significant changes in the levels of AST and ALP liver enzymes, creatinine, urea, uric acid and glucose concentrations were seen.

As a result of lupin protein administration, a decrease in serum cholesterol levels was seen in 11 of the 18 patients. The mean decrease in serum total cholesterol and LDL fraction among

respondents was 8.7% (21.19 mg/dl) and 11.1% (17.46 mg), respectively (Figure 2). No significant effect of lupin protein on serum HDL cholesterol and triglyceride levels was seen. Notably, a reduction in systolic blood pressure was seen in 58% of all subjects, and a reduction in diastolic blood pressure was seen in 50% of subjects (mean reductions of 13.2 mmHg and 7.2 mmHg, respectively).

This study is being continued for another two months and genetic tests are also being performed to evaluate the polymorphism of the ApoE gene that could partially explain the lack of response to lupin protein in some people with hypercholesterolaemia.

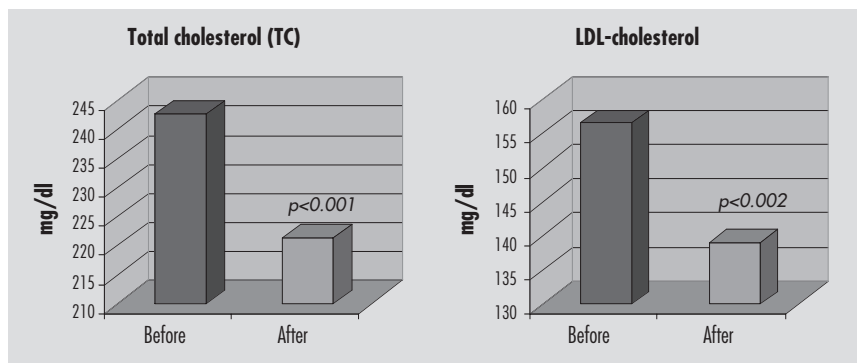


Figure 2. Effect of lupin protein ingestion (36.6 g/day) on plasma total and LDL cholesterol in 11 patients with non-familial hypercholesterolaemia.

In particular, it seems clear that subjects with the highest LDL-cholesterolaemia have the better hypocholesterolaemic response, one that is similar to what was observed in earlier studies with soy and consistent with the hypothesis that vegetable proteins may activate the so-called receptor system for LDL in the liver (4). This mechanism is down regulated in the presence of hypercholesterolaemia and thus vegetable proteins, unique among lipid-lowering treatments, can synergise effectively with drug treatments, for example, with statins, acting mainly on cholesterol biosynthesis.

Vegetable proteins are an excellent dietary product to meet the very significant interest from consumers and health authorities with the possibility of reducing the growing epidemics of coronary disease by dietary intervention. Lupin proteins that are now available in a variety of food items with excellent taste and acceptability are, potentially, a very effective addition to the list of dietary tools for atherosclerosis prevention. ■

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# Markets for French faba beans

## *Débouchés de la féverole française*

by Benoît CARROUÉE\* and Delphine BOUTTET\*\*

Translated by Pierre CASTA and Etienne de LAJUDIE (GL-Pro)

For many years, faba bean was only a marginal crop in France with less than 15,000 ha. The cultivation area was limited to clay soils, with spring types grown in the North and winter types grown in the Western Centre and the South, mainly for on-farm ruminant feed.

Yields were lower and more variable than for pea (except in maritime border regions) and market prices were 5–10% lower than for pea. As a result, seed and product supply and marketing structures remained poor and there was little interest in faba beans.

Then, at the end of the 1990s, faba bean production increased rapidly. Between 2000 and 2005, areas increased from 26,000 ha to 105,000 ha (Figure 1). This development facilitated the access to new markets and encouraged the revival of variety breeding and the registration of new crop protection products.

### The three reasons

Both UNIP<sup>1</sup> and ARVALIS<sup>2</sup> encouraged this increase in faba bean production for three main reasons:

1. Pea root rot problems were increasing. This very serious disease, due to the fungus *Aphanomyces euteiches*, spread in numerous fields in north-west France during the 1990s, often on deep loamy soil with high production potential. The only solution was to abandon pea cropping for several years. In theory *A. euteiches* can affect faba bean, but none of the different races isolated in France has ever attacked the crop. This is why faba bean has partly replaced pea in contaminated plots. In future, the advice will be to alternate pea and faba bean.

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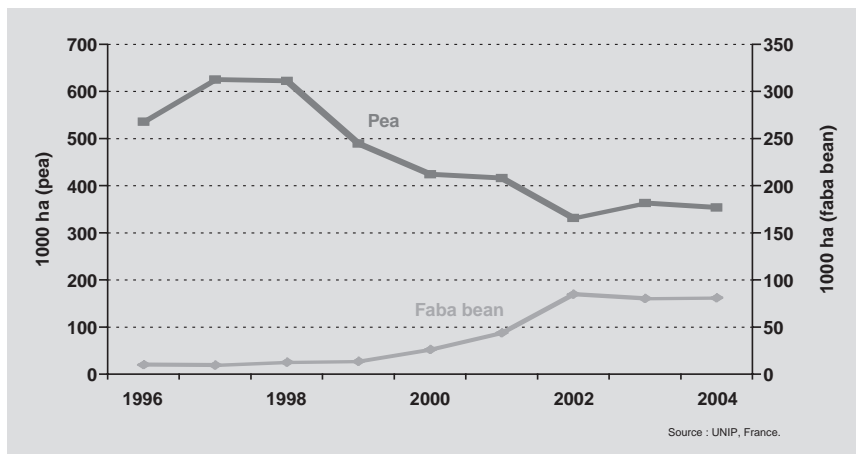


Figure 1. Pea and faba bean areas in France from 1996.

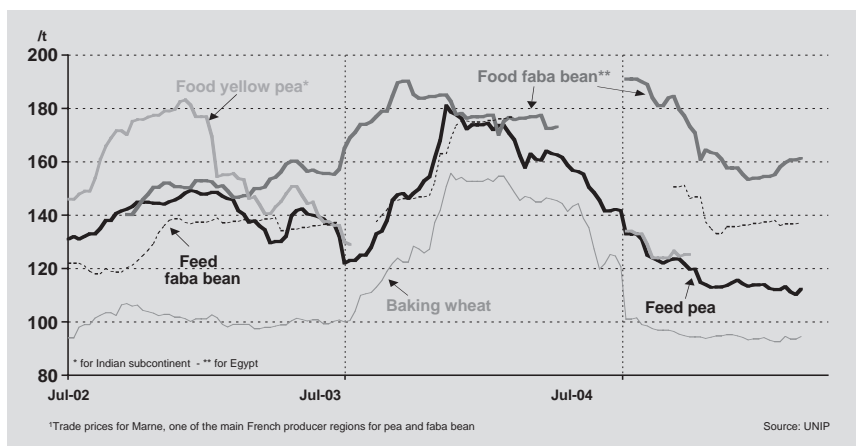


Figure 2. Pea and faba bean prices<sup>1</sup> for feed and food, compared with wheat.

2. New food export outlets were developing. From 2002–2003 there was a demand with attractive prices for food quality faba beans, mainly from Egypt. This outlet already existed but demand was met by Australian faba beans. Previously the volume of French production was too low and the quality of British production was too variable to be interesting to potential importers. However, between 2002 and 2003, while French production was increasing, the Australian harvest decreased

drastically because of drought. As France was also affected by low yield in 2003, faba bean prices reached a peak at around €200/t (Figure 2), and the Egyptian market absorbed most of the French production (145,000 t exported in 2003–2004). It is important to note that this market has very demanding quality requirements: low grain humidity (<15%) and a low level of broken, stained or *Bruchus*-damaged seeds (2–3%). In 2003, it was difficult to find enough production below these threshold levels. In

**Table 1. Faba bean outlets in France and the varieties to choose.**

Outlet	Main quality requirements	Varieties
Ruminants		All: Mélodie, Piccadilly, Lobo, Maya, Gloria, Victoria, Divine, Marcel, Karl*, Olan*, Diva*, Lady, Expresso
Pig and meat poultry	High protein content, preferably white flowered varieties	Gloria, Victoria
Laying hens	Low vicine–convicine content	Mélodie, Divine, Lady
Birds	Small seeds	Diana, Dixie
Human food: entire grains for export	No discoloration, broken seeds or <i>Bruchus</i> damage;	All, and especially Mélodie**, Divine**, Lady*
Faba bean flour (milling)	Protein concentrate (food and paper industry) High protein content	Divine, Gloria, Victoria

\* Winter types. \*\*Low vicine-convicine content can be an additional advantage.

Source: ARVALIS/UNIP, France.

2004–2005, the Egyptian demand remained high with interesting prices and France has exported about 200,000 t to this country.

3. New interest from the feed industry. Although faba bean was well known as a raw material, it could not be used in feed formulation because its production level was too low (100–200 t are needed each month to supply a local feed industry). As the peas on offer decreased, and although French and Belgian feed producers have a strong attachment to pea, the increasing quantities of faba bean on offer made them attractive to feed manufacturers. In 2003 and 2004, however, the Egyptian demand with high prices limited the faba beans available for the feed industry.

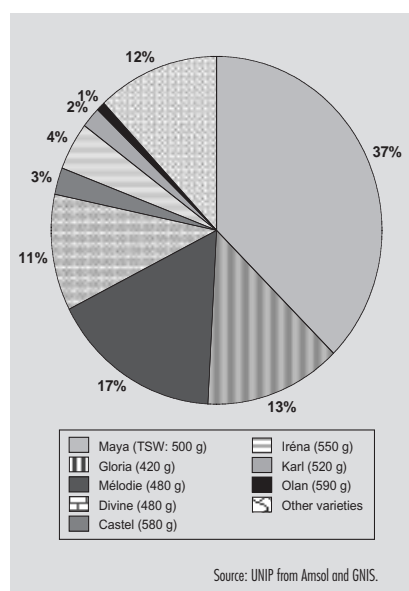
### Varieties and markets

The current development of new faba bean varieties, with enhanced nutritional quality is stimulating faba bean production. Coming from Austria, Gloria was the first white-flowered variety to be developed in France, thanks to its good agronomic behaviour and its relatively small seeds (allowing lower seed costs). For the feed manufacturer, this type of variety without tannin and with high protein content (32% on average compared with 28% for Maya, the French dominant variety) has a higher ‘interest price’ and is at least as good as pea for the pig feed industry.

A second qualitative innovation is evident in the creation of faba bean varieties with low vicine–convicine content which are being developed in France (Divine, Mélodie, Lady). Vicine and convicine are two molecules naturally present in faba bean grain that can lead to food intolerance for humans or to decreased laying performance in laying hens.

The ‘double zero’ varieties (low tannin and low vicine–convicine content) would be of greater interest for feeding poultry. Their ‘interest price’ for poultry feed formulas is as high as the ‘interest price’ of peas in pig feed formulas. These double zero faba bean varieties would not compete with peas in the same formula, as is the case for other faba bean varieties. They could also reduce risks of favism for genetically sensitive people (5% of the Mediterranean population).

The first double zero faba bean, Disco from Agri-Obtentions (INRA), was registered in the French catalogue in December 2003. However, its agronomic performance is too limited to enable significant development. In order to distinguish these new faba beans in the marketplace, the French integrated chain



**Figure 3. Market share of faba bean varieties in France in 2004 (% of certified seed sales).**

and INRA (Institut National de la Recherche Agronomique) have registered the trademark Fevita®, and this name is now limited to faba bean varieties with low tannin and low vicine–convicine contents.

### Which variety, which market?

Before choosing a faba bean variety, it is essential to know its intended market and final use. The current French outlets are very different, each with specific quality requirements (Table 1).

To maximise gross margin, it is important to take into account:

- yield/ha;
- thousand-seed weight (TSW): large seeds increase the production costs, so smaller seeds, for the same yield potential, increase the margin.

Four varieties (Maya, Gloria, Mélodie and Divine) make up 78% of the faba bean market in France (Figure 3) while others contribute much smaller percentages.

In France, approximately 75% of the faba bean area is sown with spring types. Winter faba beans are better adapted to regions with dry summers because winter types are earlier than spring types. However, winter types have limited cold resistance, and should be sown at a depth of 8–9 cm. The choice of winter type varieties is much more limited than that of spring types, which explains the interest of the farmers for spring types.

The recent increase in faba bean areas in France has taken place mainly in the northern regions, where the climate is favourable for faba beans and the farms are close to harbour facilities for export.

With this increased production, the objective is to reinforce the outlet to the Mediterranean market, through high quality standards. However, this outlet in human food will not be large enough to absorb all the production: finding the appropriate level of utilisation in the feed industry is certainly the future challenge for French faba beans. For that, the efforts undertaken to improve seed quality should be an asset. ■

<sup>1</sup>UNIP (Union Nationale Interprofessionnelle des plantes riches en Protéines): the French interprofessional organisation responsible for the promotion, the organisation of research, and information about the grain legume market.

<sup>2</sup>ARVALIS Institut du végétal: national technical institute responsible for cropping techniques for cereals, grain legumes and potato crops.

### United States pea areas keep increasing

According to USDA, pea crop areas keep increasing in the United States. More than 325,000 ha will be produced in 2005, and the main producer states are North Dakota, Montana, Washington, Idaho and Oregon. American production is partly exported within the framework of food aid programmes.

USA – Pea	2002	2003	2004	2005 <sup>1</sup>
Areas (1000 ha)	125	133	214	325
including: – green pea	85	98	164	254
– yellow pea	40	35	50	71
Production (1000 t)	194	229	518	715
including: – green pea	156	183	439	623
– yellow pea	38	46	79	92

<sup>1</sup>Estimated data (Source: USDA).

### 2005 soya harvest

The 2005 soya harvest will not reach the 2004 record (85.5 million tonnes (Mt)), but will be within the average for last five years (77 Mt). Crops suffered from drought in spite of rain in August, which is a deciding month for yield formation. The drop in production is partly compensated for by a significant stock transfer at the end of 2004/05 (8.0 Mt against 3.1 in 2003/04 and 4.9 Mt in 2002/03).

USA – Soyabean	2001	2002	2003	2004	2005 <sup>1</sup>
Harvested areas (million ha)	29.53	29.34	29.33	29.93	29.2
Yield (t/ha)	2.66	2.56	2.28	2.86	2.60
Production (Mt)	78.7	75.0	66.8	85.5	77.7
Stock transfer <sup>2</sup>	6.7	5.7	4.9	3.1	8.0

<sup>1</sup>Estimated data; <sup>2</sup>from previous harvest (Source: USDA).

### Canadian pea harvest looks promising

1410 million ha of pea were sown in 2005 (2% more than in 2004) and the harvest is looking very promising. According to recent forecasts, production could be close to the 2004 record of 3.34 Mt. For 2005, forecast production is 3.23 Mt. ■

Source: J.-P. Lacampagne, UNIP, France. Email: j.lacampagne@prolea.com

### UK pea and bean report, 2005

A cool, generally dry start to the season held back crop development and yields were variable; variety trial results indicate that commercial crops were slightly below the long-term average. For grain peas the control mean yield for 2005 was 4.58 t/ha, compared with the 5-year mean of 5.17 t/ha. For both winter and spring field beans it was the same story: winter beans yielding 4.33 t/ha compared with 4.70 t for the 7-year average; spring beans down at 3.71 t, compared with their 7-year figure of 4.15 t/ha.

Quality, for the various human consumption markets has also been variable, depending on local rainfall patterns. For many growers rain just before the pea harvest resulted in much of the grain of the large blue and marrowfat varieties showing excessive bleaching, with consequent loss of value. Field beans have also suffered, with discoloration and rather high levels of bruchid beetle infestation. Bruchid larvae develop in the grain and leave conspicuous exit holes, again devaluing the crop for human consumption. In general, the later harvested, more northerly crops have been better. ■

Source: Simon Kightley, NIAB, Cambridge, UK. Email: simon.kightley@niab.com

### Forecast production for Australia pulses

In mid-August Pulse Australia published a market overview giving projected figures for the 2005 pulse harvest (Figure 1).

Commenting in early October, John Slatter of Pulse Australia said “The weather conditions in eastern Australia have seen above average temperatures which are expected to see a reduction in the yields of some crops so I do not see any upside in our estimates”.

There continues to be strong demand from the Indian Sub Continent for chickpeas. The Middle East remains the most important export market for faba beans, with Australia facing competition from the UK and France for quality product suitable for human consumption. Access to the Indian market may yet require cleaning of peas.

The *Lupinus angustifolius* crop in Western Australia is likely to be the largest for some years (825,000 tonnes). Some 200,000 tonnes will be retained for domestic sales and on farm consumption. *Lupinus albus* for the human consumption food market (which is very colour conscious) is limited, with ruminant stock feed markets underpinning it.

Figure 1. Forecast production of Australian pulses in 2005.

	Area (ha)		Production (tonnes)	
	2004	2005	2004	2005
Field peas	280,500	290,000	224,000	364,000
Faba beans	195,000	172,000	168,000	271,000
Desi chickpeas	101,750	71,000	109,700	87,200
Kabuli chickpeas	6,500	9,250	6,500	9,300
Lentils	131,000	117,000	95,000	169,000
<i>Lupinus angustifolius</i>	625,000	742,000	490,000	942,000
<i>Lupinus albus</i>	25,600	18,500	26,000	25,900

The next update will be on the Pulse Australia web site later in October. ■

Source: Pulse Australia at <http://www.pulseaus.com.au>

### French harvest 2005: spring pea suffers while faba bean and winter pea do better

With an average national yield of 4.2 t/ha over 325,000 ha, peas were at a disadvantage this year.

The northern regions of France, where spring pea is the dominant protein crop, were the most adversely affected. In Picardie, the main region for French production, yields were 4.2 t/ha, 1.3 t/ha less than last year and 1.1 t/ha less than the mean for the years 1997–2004. In contrast, pea yields in the regions of the South were up by an average of 0.5 t/ha compared with last year.

Winter peas, earlier than spring peas, were less affected by the heatwave at the end of June but not always by the storms at maturity. Their yields were often better, but they were sometimes difficult to harvest in the north and the east.

Spring faba bean, later maturing and thanks to the cooler and sometimes wetter weather in July in the north, were able to recover their yields partially.

The quality of peas and faba beans for animal feed is good and prices for this outlet continue to be 20 €/t higher than for cereals. ■

Source: G. Dubois, UNIP, France. Email: g.dubois@prolea.com





# Towards molecular breeding for drought tolerance

## *Vers la sélection moléculaire pour la tolérance à la sécheresse*

by Jonathan CROUCH\* and Sangam DWIVEDI\*\*

**A**lthough legumes account for just 15% of arable farming land worldwide, they play a critical role in agro-ecosystems and human health, particularly in developing countries. However, investment across all sectors and regions in research and breeding of grain legume crops has fallen further and further behind that of the major cereal crop staples: rice, wheat and maize. Investment in comparative biology and translational genomics may provide a solution to this problem by leveraging progress in model species for the benefit of lesser-studied legume crops. Moreover, a shift by the plant science community to a problem-orientated research framework will be particularly beneficial for lesser-studied crops as they are often the ones that possess the best sources of beneficial traits, particularly tolerance to environmental stresses.

### **Global consortium for molecular breeding**

The Generation Challenge Program (GCP: [www.generationcp.org](http://www.generationcp.org)) is an attempt to create a global multidisciplinary and multisector problem-oriented consortium for staple tropical crops with particular reference to improving drought tolerance. This programme cuts across crops (legumes, cereals and clonal crops), disciplines (genetic resources, genomics, bioinformatics, physiology, biometrics, and breeding) and sectors (national programmes, universities, advanced research labs and the private sector) in the hope of building a more effective bridge between fundamental and applied research and thereby linking genomics innovations in the lab to impacts on productivity in farmers' fields across the developing world. Contributions to most

plant breeding goals are still to be found in the as yet largely untapped global germplasm collections. Yet the absence of efficient large-scale mechanisms for accessing and manipulating beneficial genes in these collections is a fundamental constraint to the molecularisation of plant breeding. In this article we provide an overview of the current foundations and future expectations for using comparative and translational genomics to resolve these problems, using examples from the legume crop group of the GCP.

### **Legume genetic resources are essential**

Landraces and closely related wild species possess many valuable sources of pest and disease resistance, tolerance to various environmental stresses and variation for improved agronomic characters important for increased resilience, productivity and profitability of grain legumes (2). Around half a million legume germplasm accessions have been collected by national and international genebanks around the globe. Of these, almost half are preserved in trust for the world community by the CGIAR (see [www.cgiar.org](http://www.cgiar.org) for further details). Sadly plant breeders have been reticent to make full use of these resources. Nevertheless, where successes have been achieved the impact of these novel traits has often been dramatic. However, precise and accurate assessment of genetic resources is an essential precursor for efficient maintenance and utilisation of crop-related biodiversity.

### **Evaluating exotic germplasm for plant breeding**

The development of core collections has been shown to be a particularly useful strategy for providing crop breeding programmes with a systematic yet manageable entry point into global germplasm resources. However, these are generally restricted to the contents of individual genebanks and

often biased by the nature of phenotypic descriptors used to design them. Thus, over the past two years the GCP has funded the development of core collections based on molecular genetic analysis of subsets of germplasm from all major collections across the world for 22 cereal, legume and clonal crops. These will provide definitive entry points for plant breeders interested in any trait, powerful resources for association mapping of agronomic traits and a critical starting point for future investments by the GCP and others in functional genomics investigations of those traits. In turn, these scientific advances should provide the necessary tools required for effective manipulation of these traits in plant breeding programmes (for further details see [www.generationcp.org](http://www.generationcp.org)).

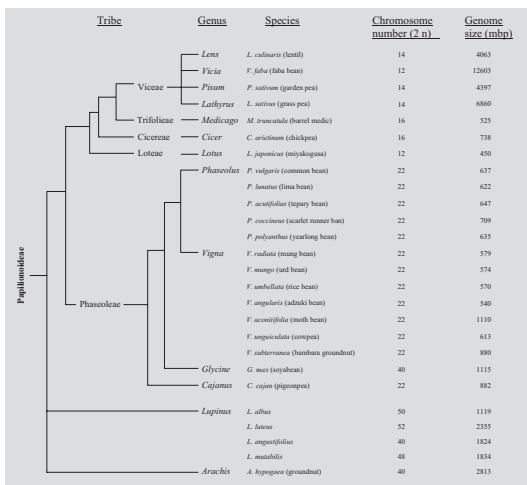
### **Comparative and translation genomics for molecular breeding**

Comparative genomics research in the 1990s demonstrated a high level of similarity between the order of genes (so-called synteny) across diverse cereal crops (4). These findings supported a massive investment in the genomics research of the model system *Arabidopsis* (and later in the legumes, *Medicago* and *Lotus*), with the expectation of substantial direct spillovers for all crops. There is no doubt that this has exponentially accelerated our ability to generate new knowledge across the plant sciences (3). Unfortunately, the spillover model has proved to be inadequate to drive practical outputs for plant breeding programmes in those crops at the level of specific agronomic traits. This is partly due to the high level of divergence within crop groups such as the legumes (Figure 1). Consequently there has been a resurgence of investment in crop genomics and an increasing acknowledgment from the model system community of the need to work translational genomics. However, most of these efforts have now

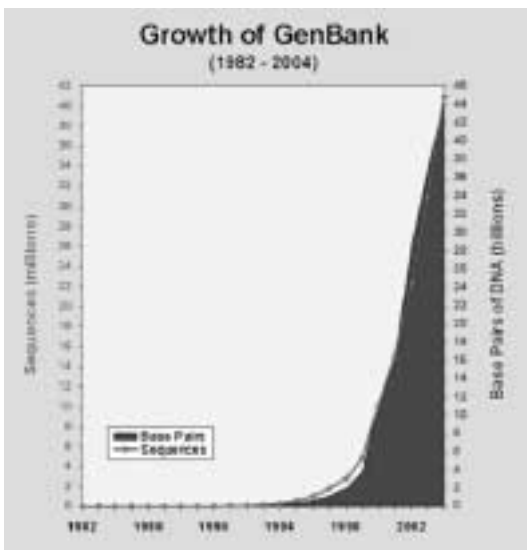
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\*\*International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India.



**Figure 1. Relationships between legume crops and model species: dendrogram showing the phylogenetic relationship between the legume model genomes (*Medicago truncatula* and *Lotus japonicus*) and major temperate (*Lens*, *Vicia*, *Pisum*, *Cicer* and *Lathyrus*) and tropical (*Phaseolus*, *Vigna*, *Glycine*, *Cajanus* and *Arachis*) legume crops together with their chromosome numbers and genome sizes.**



**Figure 2. Growth of GenBank: histogram showing the increase in sequences available in the public domain via GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/>).**

shifted away from gene order studies (so-called structural genomics) to gene sequence-based analysis and gene function validation. This approach lends itself much more readily to the development of gene-based markers that will have direct value for plant breeding programmes.

### Dissecting and manipulating legume physiology

The GCP has adopted a defining focus on gene-based research amongst crop groups, with a particular emphasis on genes shown to be involved in drought tolerance.

In order to make maximum use of knowledge from the genotype-rich model systems we consider it essential to invest heavily in detailed genomic analysis of phenotype-rich crops renowned for their drought tolerance, such as cowpea for the legume crop group (plus pearl millet for the cereals and cassava for the clonal crops). Thus, the defining feature of the GCP is the development of gene-based tools for helping plant breeders improve the efficiency of identifying useful accessions in global germplasm collections and of transferring valuable traits into their breeding populations.

Modern advances in genomics offer unprecedented abilities to dissect the genetic basis of complex agronomic characters and study the roles of individual genes in specific metabolic pathways. However, rebuilding the biological significance of these building blocks and understanding how they interact with each other (epistasis) and their environment (genotype-by-environment interaction) will be a fundamental precursor to their effective manipulation in plant breeding. Developments in whole plant physiology modelling now offer new opportunities for understanding this multidimensional basis of complex traits. Advances in simulation analysis promise to assist plant breeders to devise the most effective breeding system for manipulating complex traits in a rational and targeted manner (1).

assist plant breeders to devise the most effective breeding system for manipulating complex traits in a rational and targeted manner (1).

### Transgenic technologies in candidate gene validation

Identifying and isolating individual target genes is an extremely time consuming and expensive process. Yet when unique genes of substantial effect are identified, this approach will be important in order to achieve broad-ranging impacts beyond species barriers. The GCP will generally leave others to focus on transgenic product

development using such genes.

In contrast, where the emphasis is on developing gene-based tools (for allel mining and molecular breeding), there are new (so-called RNAi) approaches based on interrupting the expression of individual candidate genes that can be used when only the partial gene sequence has been elucidated (5). These approaches offer for the first time, validation of gene function on a scale and throughput that may be able to keep pace with large-scale sequencing programmes (Figure 2).

### Future prospects

The genomics revolution, led by progress in the model species, has had a profound impact on all aspects of plant science research. However, as yet there has been only limited translation of that knowledge into new skills for tropical plant breeding programmes. This is partly due to the inevitable unveiling of unexpected levels of complexity as we have delved more deeply into the genetic basis of complex traits and their expression in complex target environments. This will change dramatically with the transition to gene-based approaches that now provide a direct link between the outputs of fundamental research and the development of tools that will help plant breeders manipulate complex traits such as drought tolerance. It is the primary goal of the GCP to harness these tremendous new opportunities for the benefit of resource-poor farmers across the world – by providing the necessary genetic variation and a viable mechanism for a more rational and targeted design of seed-based products (6) that provide increased economic potential, enhanced environmental resilience and reduced need for agrochemical inputs. ■

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# GRAIN LEGUMES



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*Le magazine de l'Association Européenne de Recherche sur les Protéagineux*

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Biscuits, drinks and muffins made with lupin ingredients (Photo Fraunhofer IVV, Germany)

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*Biscuits, boissons et muffins à base d'ingrédients de lupin (Photo Fraunhofer IVV, Germany)*

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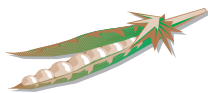
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*The AEP is an associative network of persons with interests in grain legume research (peas, faba beans, lupins, chickpeas, lentils, dry beans, etc.) to favour the exchange of information and multidisciplinary collaborations (Conferences, publications, workshops, joint projects). It aims both to strengthen the research works and to enhance the application of research into the integrated chain of grain legumes.*



## UNIP

French Interprofessional Organisation of Protein Crops  
Union Nationale Interprofessionnelle des plantes riches en Protéines

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*The UNIP is the representative organisation of all the French professional branches of the economic integrated chain of grain legumes. It provides information about pulse production, utilisation, and the market and it coordinates research works related to grain legumes in France, especially peas, faba beans and lupins for animal feeding.*



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*The PGRO provides technical support for producers and users of all types of peas and beans. Advice is based on data from trials sited from Scotland to the South West of England and passed to growers and processors through technical bulletins and articles in the farming press.*



## APPO

Belgian Association for Oilseeds and Protein crops  
Association pour la promotion des protéagineux et des oléagineux

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*The APPO is the representative organisation of Belgian growers of oilseeds and protein crops, especially rapeseed, peas and faba beans. The main tasks are experimentation, giving advice to producers, providing technical and economic information through meetings and mailings and encouraging non-food uses of vegetable oil.*



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*UFOP is the representative organisation for German producers of oil and protein crops. It encourages professional communication, supports the dissemination of technical information on these crops and also supports research programmes to improve their production and use.*



## CRA-W

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*A broad research topic of the Animal Production and Nutrition Department deals with the utilisation of lupin and pea seeds in animal feeding (ruminant, monogastric and poultry) in terms of nutritional value, environmental benefits, protein utilisation and economic aspects. The research is also concerned with the development of legume silages, seed treatments prior to feeding and seed processing for non-food uses.*



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*Pulse Canada is a national industry association. This organisation represents provincial pulse grower groups from Alberta, Saskatchewan, Manitoba, Ontario and the pulse trade from across Canada who are members of the Canadian Special Crops Association. Pulse crops include peas, lentils, beans and chickpeas.*

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