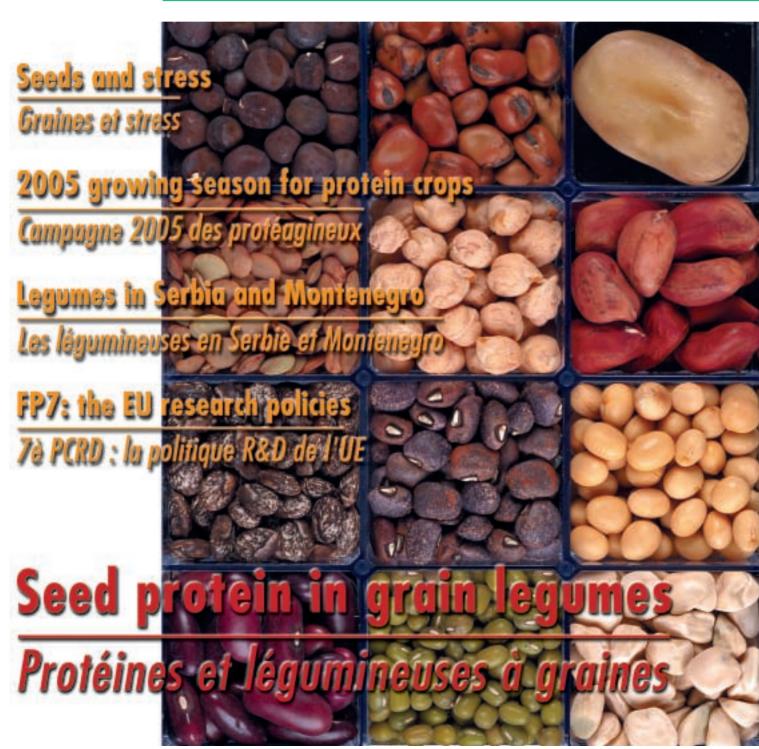


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The magazine of the European Association for Grain Legume Research Le magazine de l'Association Européenne de recherche sur les Protéagineux

ISSUE No. 44

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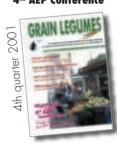
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Increasing protein supply

Protein is one of the key assets associated with grain legumes: not only are they self-sufficient in nitrogen supply, but the rich protein content of their seeds makes them attractive for animal feed and the reason why they were developed in Europe in the 1980s for the feed industry. How do we enhance protein quantity and quality in grain legume seeds? This special report contributes to the discussion with different analyses and strategies.

The past two growing seasons were not favourable for cereals and grain legumes, as reported in our crops section. However, analysis of the 2005 growing season has provided valuable information on traits that have to be enhanced, such as resistance to high temperatures at crucial periods in the growth cycle, or standing ability to resist heavy rain. Research and development activities need to produce crops which are flexible and adapted to expected and unexpected climatic fluctuations.

On a seasonal note, the Editorial Board wishes you a Happy New Year. We hope that 2006 will provide you with opportunities for personal success, and also opportunities to interact further with Grain Legumes magazine!

Please feel free to contact us with your views and to propose news or articles. Your magazine is a unique 'crossroads' where different experiences and expertise come together and can be disseminated to a diverse audience.

Anne SCHNEIDER

Managing Editor

Carte blanche

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(Á Ramos Monreal)

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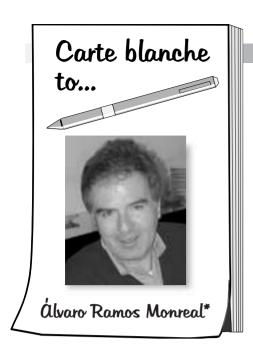
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New international plant genetics resources treaty

The International Treaty on Plant Genetics Resources for Food and Agriculture (described in more detail in the News section of the AEP website at www.grainlegumes.com) has finally been reached after twenty-three years of negotiations. For the last six years, when negotiations were especially intensive, I have taken part as a representative of the Spanish National Commission for Genetic Resources.

This Treaty is of major significance because, for the first time, all the countries (Contracting Parties) that signed and ratified the Treaty are obliged to introduce into their legislation rules for the conservation, exploration, collection, characterisation, evaluation, documentation and sustainable use of Genetics Resources for Food and Agriculture.

Also for the first time, all the Contracting Parties will take measures to protect and promote Farmers' Rights as the creators of hundreds of thousands of landraces around the world. The Contracting Parties agreed to form a Multilateral System to facilitate access to plant genetics resources for food and agriculture and to share, in a fair and equal manner, the benefits arising from the utilisation of these resources.

The Multilateral System covers the plant genetics resources listed in Annex 1 of the Treaty (35 food crops, calculated as 80% of human food needs, and 29 forages) which are in the management and control of the contracting parties and in the public domain. For the first time benefits arising from their use, for example through commercialisation, will have to be paid to the Multilateral System. Some countries were very reluctant to include more crops because they expected to get more benefits from bilateral negotiations. Crops that provide the vitamins most needed in our diets are very limited in number on the present list, but the list is open and may be more complete in the future.

The development of the Treaty is presently under multiple negotiations to agree on a common Standard Material Transfer Agreement, which is at the heart of the Multilateral System, as well as to agree on Rules of Procedure, Financial Rules, Procedures to Promote Compliance of the Treaty and so on. All these mechanisms will have great importance for the effectiveness of the Treaty and on genetics resources for food and agriculture.

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EU policies and FP7

The current political vision of the EU is reflected by the Lisbon objectives and the Lisbon Declaration (March 2000) includes the objective to "become the most competitive and dynamic knowledge based economy in the world capable of sustainable economic growth with more and better jobs and greater social cohesion".

The EU policies are developed through a series of programmes whose implementation is the responsibility of the European Commission.

From 2007, these programmes will be re-organised into further major programmes:

- Framework 7 (for research issues)
- CIP Competitiveness and Innovation Programme (which will include part of the previous FP6 innovation)
- Structural funds (for innovation)
- Education Programmes

These programmes target the Lisbon objectives and reflect the concept of the Triangle of Knowledge "Education, Research and Innovation" (raised in the Kok report 'Facing the Challenge: The Lisbon strategy for Growth and Employment' November 2004).

The European Research Area (launched in FP6) is a target to strengthen EU research and excellence, and to avoid duplication by overcoming fragmentation and stimulating a collaborative approach for EU research.

Frontier Research and the ERC will boost basic research

Backed by a group of Nobel prize winners, and formalised since a Dublin Conference in February 2004, basic research will be covered by a sub-part of FP7: the 'Frontier Research' programme. In addition, the Marie Curie programme will be expanded (fellowships and network).

Frontier Research is an 'investigator driven' approach (no consortium). The evaluation will be based on a competitive peer-review and the only evaluation criteria will be scientific excellence.

Insert 1

FP7 timetable

- From 2005 to mid-2006: Strategic phase: Proposal from the Commission for FP7 on 6 April 2005; Consultation and co-decision Parliament/Council; Amendments by the European Parliament; Consultation with the Economic and Social Committee, the Committee of the Regions, the EU Parliament, the European Council (Coreper); Definition of Research priorities and Rules of participation; Distribution of budget (project in discussion).
- Second part of 2006: Final administrative stages in the co-decision process for adoption of FP7.
- End 2006: Programme planning phase; End of FP6; Launching of FP7.
- Early 2007: Call for proposals
- 2007-2013: Implementation Phase

More information at the CORDIS web site

The quantity of information available on FP7 is considerable, but it is clearly presented on the CORDIS website, Towards FP7 — your gateway to the preparation of the Seventh Framework Programme at http://www.cordis.lu/fp7/home.htm

From this home page there are clear links to all the associated relevant information. The FP7 Frequently asked Questions (FAQs) have been designed and structured in the context of the preparation of the Seventh Framework Programme (FP7) and the answers rely on the formal proposal of the Commission to the Council and Parliament on 6 April 2005 and on other official documents published until now.

COOPERATION	Health Food, Agriculture and Biotechnology Information and Communication Technologies Nanosciences, Nanotechnologies, Materials and new Production Technologies Energy Environment (including Climate Change) Transport (including Aeronautics) Socio-economic Sciences and the Humanities Security and Space
IDEAS	European Research Council
PEOPLE	Marie Curie Actions
CAPACITIES	Research Infrastructures Research for the benefit of SMEs Regions of Knowledge Research Potential Science in Society Activities of International Co-operation
	ons of the Joint Research Centre ear research and training activities

Table 1. Structure of the Seventh Framework Programme of the European Community (EC) (2007–2013) and Euratom (2007–2011).

Grants (not contracts) will be provided for individual teams, which will decide on their own the need for collaborations with other teams or countries. The European Research Council (ERC) is now established with 22 eminent men and women who have been appointed to be the founding members of the Scientific Council of the European Research Council (ERC), chaired by Lord Patten of Barnes, Chancellor of Oxford and Newcastle-upon-Tyne Universities. The Scientific Council will be an independent body representing the interests of science and research at the very highest level. The NSF (Noble Foundation in the USA) seems to be a source of inspiration for the implementation of Frontier Research and ERC.

Technology Platforms will enhance the impact of research

FP7 is suitable for companies that have a more long-term vision and plan. One of its aims is to encourage industry to support more research (2/3 of the '3% objective' should come from the private sector).

The Lisbon Action Plan proposes to "support industrial competitiveness through major European Technology Initiatives", and "translate Strategic Research Agenda (of the Technology Platforms) into the Thematic Priorities of Framework 7".

Structure and implementation of FP7

The FP7 will be organised around four programmes (Cooperation, Ideas, People, Capacities) (see article GLM No. 42 page 6 and see Table 1). The Cooperation programme covers nine themes. One of these themes is 'Food, agriculture and biotechnology', which "aims at building a European 'knowledge based bio-economy' to answer the growing demand for safer and healthier food as well as for sustainable use and production of renewable bio-resources".

One of the innovations of FP7 is a specific theme on Socio-economic Sciences and the Humanities.

The instruments used in FP7 will be the same as in FP6 (following a recommendation by external panels).

Continued overleaf...



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Covering all the nine themes, Era-Net will be maintained (as in FP6 for SSA and CA) and ERA-net plus will make it possible to implement Article 169 with new initiatives.

Competitiveness and Innovation Programme (CIP)

CIP will include the part "innovation" previously in FP6. It will include:

- Entrepreneurship and Innovation programme (60% of the CIP budget): access to finance for start-up and growth of SMEs, SME cooperation, Innovation including eco-innovation in enterprises, Entrepreneurship and innovation culture, Economic and administrative reform.
- ICT (Information and Communication Technologies) policy support programme.
- Intelligent Energy-Europe Programme.

Source: Anne Schneider (Email: a.schneideraep@prolea.com) from sources such as Hyperion Ltd, European Commission (Conferences and

13% objective: the EU Council has decided to aim for 3% of GDP for the investment in research by 2010.

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



The Madrid 2005 Dissemination Event informed a wide ranging audience about the current grain legume situation, demonstrating that these crops and products are recommended for realising more sustainable European agriculture, and that current research on these crops is promising and ongoing, especially in the area of plant genetics and breeding.

The book of abstracts and slides is freely available at AEP headquarters (aep@prolea.com — and at http://www.grainlegumes.com/default.asp?id_biblio=392

Grain legumes for **European agriculture** and environment: benefits and prospects **GL-Pro dissemination event -**



Brussels, 3-4 May 2006

This event will provide the main opportunity to disseminate and discuss the results of the techno-economic and environmental analyses carried out within the GL-PRO network during the three year EU concerted action.

Contact: AEP and GL-PRO www.grainlegumes.com/gl-pro

Food Legume Research Conference in India

The 4th International Food Leaume Research Conference (IFLRC-IV) was held from 18 to 22 October 2005 in New Delhi in India. It was organised by the Indian Society of Genetics and Plant Breeding, the Indian Council of Agricultural Research and the Indian Agricultural Research Institute. About 500 participants including 100 international experts were gathered in sessions dealing with a wide range of scientific areas

Seeds and stress: new approaches for discovery

Graines et stress : nouvelles stratégies d'investigation

by Emilio CERVANTES*

iambatista Vico published his book entitled 'Principi di scienza nuova' in Naples, in 1744. At the beginning of the second section, the author writes: "Man, by the undefined nature of the human mind, when this submerges into ignorance, makes of himself the rule of the Universe". In this sentence, the author recognises the risks of personification, that is, the attribution of human attributes to non-human beings. Interestingly, more than two hundred and fifty years later, the scientific literature offers many examples of personification. In the long term, personification may result in a poor and restricted view of the world around us, by which we will think of nature in general, or some of its elements, as having characteristics of human beings that only we humans, have. On the other hand, many of the words and concepts that exemplify personification give very graphical representations of the behaviour of organisms which contribute to advances in their understanding. Thus, language in science is a two-edged sword that we need to use with precaution.

The meaning of stress

The term 'stress' was used first in human physiology meaning a status of acute tension of the organism, forced to mobilise its defenses to confront a menacing situation. It was introduced by the Hungarian physiologist Hans Selye (1907-1982) and since then, it has been used widely in the biological sciences as well as introduced in the colloquial language of most countries throughout the world. Initially, the term 'stress' was intended to be applied to human

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behaviour, but it is now used widely in the plant sciences. We do not know whether or not plants really 'suffer', or go through any status similar to 'acute tension' or even if an event as frequent for humans as a 'threatening situation' has, for plants, a meaning outside that of personification. Nevertheless the use of the term and the investigation of the 'stress' has contributed considerably to our understanding of biological processes in plants.

One of the main characteristics of living beings is their capacity to interact with others and with the environment. For this, they have developed biochemical mechanisms adapted for sensing variations in the environment that constitute the stimulus, and integrating diverse stimuli in the elaboration of a response (Figure 1).

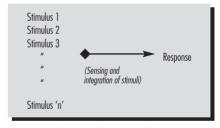


Figure 1. Mechanism of response to the environment.

In biology, stress was described first in terms of hormonal or endocrine modifications resulting in the activation of defense-response mechanisms in humans. This is of particular interest because the analysis of the defense–response mechanisms activated under stress reveals essential aspects in the mechanisms of sensing and integration of stimuli and elaboration of responses. Therefore, whether or not the term is appropriate, it opens the door for the analysis of signal-transduction processes that are essential components of biological processes.

New environmental response mechanisms

Seeds are very interesting structures. They are specialised in two activities that they are able to do better than humans. First, they are specialised in resisting harsh environmental conditions and changes. A human being may resist without drinking water for a few hours, or for a few days at the maximum, while a seed may resist years, often without any detectable modification. Changes in the environmental variables that can put humans or animals under severe stress are tolerated easily by seeds without any difficulty or major alteration. Even more surprising than their capacity to resist stress is the capacity of seeds to sense variations in the environment and to integrate these sensations to give a response such as germination, or not to respond at all. From this point of view, germination is a stress response, and the seeds are structures specialised in survival from stress.

In the plant's life cycle, germination fixes the time and place for further development. It marks the transit between a dynamic structure, the seed, and a static organism, the plant. Being of crucial importance, germination must be regulated tightly. The seed possesses sensing mechanisms that allow it to germinate only under favourable conditions.

Light, temperature, water, nutrients, salts, metals and other physical factors of the environment may affect seed viability and germination. Many important advances in plant physiology are the result of the study of the responses of seeds to these factors, but now with genomics, understanding the process of seed germination has, as an obligatory first step, the analysis of seed germination in the model plant, Arabidopsis thaliana. The comparison of the process in the model with other plant species (such as legumes) is the second stage of the investigation. It is clear today, that even in the simplest model plant, Arabidopsis, germination is not a simple process and it reveals new and unexpected features, among which there may be new mechanisms for sensing environmental variations and their integration in a developmental process. In plants such as legumes with larger genomes, germination will be a fascinating process full of surprises and unexpected results awaiting future research

Unknown genome sequences mobilised

In a recent experiment designed to identify and clone nucleotide sequences expressed during seed germination in Arabidopis, a total of 50 different sequences were cloned. Among these sequences some corresponded to known genes of Arabidopsis involved in transcription, antioxidant defenses, hormone responses. mitochondrial biogenesis and cell cycle. Others corresponded to Arabidopsis sequences in BAC clones that have not been annotated as genes. A third group, and an important proportion of the sequences, were not like available sequences from the Arabidopsis genome, but were more like DNA sequences from other organisms, such as fish species, and some of them may encode transposons. A number of the sequences isolated showed no homology with any sequences in the databases, suggesting that previously unknown genome sequences are mobilised during the process of seed germination. This work (2) has already been presented at several meetings and questions are being raised as a result of it. First, do the new sequences really correspond to the Arabidopsis genome? Secondly, why are there so many new sequences, and why are those new sequences expressed during germination?

Many of the new sequences found have been re-amplified from Arabidopsis DNA or used as probes in northern and southern blots. Therefore, to answer the first question, they do indeed correspond to the Arabidopsis genome. The answers to the other questions require more speculation. One possibility is that the constant light condition used for seed germination in the initial cDNA-AFLP experiment could be the source of an additional stress superimposed on the natural stressful situation of germination and resulting in an exceptional genomic response. Since the pioneering work of McClintock (4), transposons are known to be involved in dynamic responses of the genome when submitted to external changes (stress). Recently, it has been shown that many non-coding RNAs may be expressed in a variety of tissues and developmental conditions. Among them some may be precursors of micro RNAs, whose roles in differentiation processes have been demonstrated recently both in plant and in animal systems. Also, a large population of retrotransposons has been described recently in oocytes and their role in the regulation of gene expression during the early stages of vertebrate development has been suggested (5).

The unfolded protein response

Many trends in biology originate from work done on mammalians and then expand to studies on plants. Recently the Unfolded Protein Response (UPR (6)) is acquiring importance as a mechanism controlling many cellular processes in mammals. Basically, UPR departs from the existence in the cell of a series of mechanisms that recognise the signals from unfolded proteins and use them as a trigger to initiate developmental responses including changes in the organelles and apoptosis. During long storage periods, seeds accumulate unfolded and degraded proteins, and immediately upon imbibition important organellar changes accompany the cellular elongation that results in germination. Upon germination, vascular bundles need to be formed and for this developmental process, apoptosis is necessary. Evidence has accumulated in recent years that plant hormones interact with each other and with central pathways of cellular metabolism. Free radicals are produced during active metabolism and respiration and are mediators of the stress response. Hormones interact with the machinery of protein degradation in multiple ways (1, 3). Seeds are an excellent system in which to investigate these interactions. Nature has more richness than we think and seeds are a good example of how rich and varied processes may still remain undiscovered. As Heraclitus said: "Nature loves to hide".

⁽¹⁾ Dharmasiri, N. et al. (2005). Nature **435** (7041), 441–445.

⁽²⁾ de Diego, J. G. et al. (2005). J. Plant Physiol. (in press).

⁽³⁾ Kepinski, S. and Leyser, O. (2005). Nature **435** (7041), 446–451.

⁽⁴⁾ McClintock, B. (1984). Science 226, 792.

⁽⁵⁾ Peaston, A. E. *et al.* (2004). Developmental Cell **7**, 597–606.

⁽⁶⁾ Schroder, M. and Kaufman, R. J. (2005). Ann. Rev. Biochem. **74**, 739–789.

PhD thesis

Breeding for resistance to broomrape (*Orobanche crenata*), ascochyta blight (*Mycosphaerella pinodes*) and powdery mildew (*Erysiphe pisi*) in peas using traditional breeding and molecular markers*

Mejora genética del guisante para resistencia a jopo (Orobanche crenata), ascoquitosis (Mycosphaerella pinodes) y oidio (Erysiphe pisi) mediante mejora clásica y marcadores moleculares

by Sara FONDEVILLA**

wo single recessive genes, er1 and er2, for resistance to powdery mildew have been identified in pea. In the present thesis the histological mechanisms involved in the resistance conferred by these genes have been investigated. In accessions carrying gene er1, epidermal cell penetration was prevented and very few haustoria or colonies were formed. Under controlled conditions, er1 conferred complete or almost complete resistance. Under field conditions these accessions developed a low level of disease. Resistance in line JI2480 (carrying er2) increased with temperature and leaf age, and complete resistance was expressed only at 25°C or in mature leaves. Resistance was based mainly on post-penetration cell death, complemented by a reduction in penetration success in mature leaves. In addition, 67 accessions of different Pisum species were screened for resistance to E. pisi. P. fulvum accessions were highly resistant, but moderate resistance was identified in P. sativum ssp. sativum var. arvense, P. sativum ssp. elatius and P. sativum ssp. abyssinicum. Both pre-penetration and post-penetration resistance reactions against powdery mildew, including a hypersensitive response, were identified. Resistance in P. fulvum accessions P651 and P660 was controlled by a dominant

single gene. Four RAPDs were identified in the coupling phase and two in the repulsion phase with this gene.

A population consisting of 111 RILs (Recombinant Inbred Lines) derived from a cross between a *P. sativum* spp. *syriacum* accession partially resistant to *O. crenata* and *M. pinodes* and the susceptible pea cv. Messire were analysed and a linkage map has been developed. This map covers 1214 cM and contains three morphological, one isozyme, 12 STS and 235 RAPD markers distributed in nine linkage groups. Of these, six groups have been assigned to chromosomes using markers common to the consensus pea map. One QTL for broomrape resistance, explaining 19% of phenotypic variation, was found in chromosome 4. Four QTLs for resistance to *M. pinodes* (68% of the resistance) were also identified in this population. In addition, several *Pisum spp.* accessions with moderate levels of resistance to *M. pinodes* were identified under field conditions and their resistance confirmed under controlled conditions against different isolates of the pathogen from Japan, Canada, Spain, France and Poland.

*PhD thesis June 2005, Genetics Department, University of Córdoba, Spain.

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EVENTS

FEBRUARY 22, 2006

Open day of the Second Annual GLIP Meeting and the General Assembly of the GL-TTP INRA, Montpellier, France.

Web: http://www.eugrainlegumes.org/meetings/GLIP_second_annual_meeting.htm

MARCH 25-29, 2006

1st Mediterranean Congress on Biotechnology
Hammamet City, Tunisia. Web: http://www.fmcb.africa-web.org/presentation.htm

APRIL 9-13, 2006

3rd International Conference on Legume Genomics and Genetics
Brisbane Convention and Exhibition Centre, Queensland, Australia. Web: www.iclqq3.org/

MAY 3-4, 2006

Grain legumes for European agriculture and environment: benefits and prospects GL-Pro dissemination event, in association with GLIP Brussels, Belgium. Web: www.grainlegumes.com/gl-pro

JULY 3-6, 2006

Aschochyta 2006, 1st Aschochyta Workshop on Grain Legumes
Deadline for registration and abstracts February 28, 2006. Email: aep@prolea.com

JULY 22-26, 2006

7th European Nitrogen Fixation Conference Aarhus, Denmark. Web: www.7theuronfc.dk

SEPTEMBER 4-6, 2006

IX ESA Congress

Warsaw, Poland. Web (under construction): www.esagr.org/





Optimised processes for preparing healthy and added value food ingredients from lupin kernels, the European protein-rich grain legume

October 2005, English, 202 pages Anna Arnoldi (Ed.) Aracne editrice S.r.l., Rome ISBN 88-548-0267-0

This book contains the proceedings of the Final Conference of the Healthy-Profood Project (see the special report in *Grain Legumes* No. 43, 13–21). The conference was organised on 9–10 November, 2005 at the Visconti di Vimodrone Palace, Milan to present the most relevant results of the project to the international public.

Price: free **Order:** AEP (aep@prolea.com)

OCTOBER 25-27, 2006

Faba bean 2006 — International workshop on faba bean breeding and agronomy. Córdoba, Spain. Registration extended until February 28, 2006. Email: anam.torres.romero⊕juntadeandalucia.es

NOVEMBER 12-16, 2007

6th AEP Conference and Final GLIP meeting. Lisbon, Portugal. Email: aep@prolea.com



Seeds of diverse Pisum germplasm.

eeds of peas and faba beans have a mixed profile of raw materials, providing both energy and protein to feed humans and animals. Protein was the major reason for their development especially in Europe. Where the advantages of local supply are recognised, and as the deficit in material rich in protein is still rising in the EU (76% in 2003/4), increasing EU production of grain legumes would have a positive effect on EU supply. Crop yield and seed protein are the two key parameters for increasing this protein supply, and the enhancement of seed protein should be both quantitative and qualitative (acid profile amino and digestibility).

The first article describes the cultivated material, analysing the variations of protein content in registered European pea cultivars and in different environments. A better understanding of the complex relationships between crop yield potential, plant carbon costs and protein content will then enable us to point to the best strategies for crop improvement and highlight the existing potential for genetic progress in grain legumes.

Increasing protein content and yield simultaneously by genetic improvement through breeding is the major challenge being addressed. Current knowledge and ongoing work on seed content and its control in peas and faba beans are described, showing that unravelling the genetic and environmental control mechanisms will be of key importance to exploiting the available genetic variability and accelerating seed improvement.

In addition, possible scenarios for the feed industry are simulated using modelling tools to test, in the current context, the economic value of improved peas for the poultry feed market.

es graines de pois et de féveroles sont des matières premières à profil mixte, apportant énergie et protéines pour nourrir les êtres humains et les animaux. La protéine a été le facteur majeur de leur développement surtout en Europe. Dans un contexte où l'intérêt de la production de proximité est reconnu et où le déficit de l'UE en matières riches en protéines continue de croître (76% en 2003/4), un élément de réponse positive est d'augmenter la production de protéagineux en Europe. Le rendement de la culture et la teneur en protéines des graines sont les deux clès d'entrée à améliorer pour augmenter l'approvisionnement en protéines, et l'augmentation de la teneur en protéines doit être quantitative et qualitative (profil en acides aminés et digestibilité).

Le premier article décrit la situation actuelle en étudiant les variations de la teneur en protéines au sein des variétés de pois inscrites en Europe et dans plusieurs environnements. Mieux appréhender les relations complexes entre le potentiel de rendement d'une culture, les coûts en carbone pour la plante et la teneur en protéines, nous permet ensuite d'analyser les stratégies d'amélioration des cultures et de voir la marge existante pour le progrès génétique chez les légumineuses.

Améliorer simultanément la teneur et le rendement de la protéine par la sélection variétale est le défi majeur traité ici. Sont résumés les connaissances actuelles et les travaux en cours sur la compréhension de la teneur en protéines et de son contrôle, pour le pois et la fève-féverole. La dissection des mécanismes régulateurs génétiques et environnementaux sera la clé pour exploiter la variabilité génétique existante et accélérer l'amélioration des variétés cultivées.

De plus, les scénarios de comportement du secteur de l'alimentation animale sont simulés pour tester l'intérêt économique, dans le contexte actuel, de pois améliorés pour le secteur de la volaille. ■

Protein content of registered European cultivars: variations among cultivars and trends over time and location

Teneur en protéines des variétés inscrites en Europe : variations en fonction des variétés, des années et des lieux

by Véronique BIARNÈS*

years and locations (1) and in the European Field Pea Network in 1998–99 (nine locations in seven western European countries (2)) all showed that the effect of environment is the first source of variation for seed protein content. For example, in the Network trials over two years, the seed protein content for pea cv. Athos ranged from 19% to 28%.

The variety effect for protein content is usually significant. In French trials for registration there is generally a difference of 2% or 3% between the varieties with the lowest and highest protein content. In the Network trials the difference was also around three percentage points between cv. Athos which is rich in protein (25–26%) and the cv. Eiffel or Swing which are lower in protein (22–23%).

Strong environment effect but ranking of varieties very stable

In contrast, there are few genotype x environment interactions for seed protein content, but the genotype x environment interaction effect can be as important as the genotype effect (1). The ranking of varieties for seed protein content is very stable from one site to another. In all environments the same varieties are found at the top and bottom of the ranking. For intermediate varieties, there can be inversions but the differences among varieties are often around 0.5 percentage points of seed protein content, which

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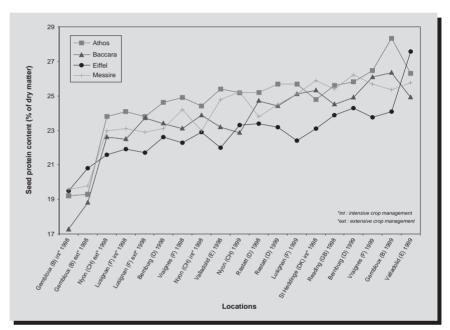


Figure 1. Seed protein content of four varieties in the European Field Pea Network 1998–99.

corresponds to the level of precision of the measure. Then, they are not significantly different. The same observations were made in most of the Network trials in 1998 and 1999 over different climates (Figure 1), except at Gembloux in 1998, where bad weather conditions at sowing and at emergence may have affected the nitrogen fixation and growth of plants and finally the yield and the protein content. Some plots were submerged by water. This can explain the very different ranking of varieties compared with one of the other sites. In 1999 at Valladolid very dry conditions during the spring may have affected the nitrogen nutrition and the seed protein content and may have changed the ranking of varieties. It was clear that the variety Messire had some bad results in some sites and good ones in other sites. This variety also had irregular yield.

Year and location effects important when drought stress before flowering

There is generally little difference in the ranking of varieties from one year to another, except in a very dry year. In the French study over a number of years (1), the genotype x year interaction was significant when the year 1996 was included in the data. This year was marked by a significant drought which had begun very early in the spring and which could have disturbed the nitrogen nutrition and especially the nitrogen fixation by the plant. In this particular year, the ranking of varieties was completely different from the other years. In the European Field Pea Network, the seed protein content was higher in 1999, a very favourable year, than in 1998, which was a hot dry year, but the ranking of all the varieties for each year

was exactly the same. It is possible to take the values and relate them to a control in a given year and for each country. These are then stored in a database (see insert).

The genotype x location interaction was significant in half of the situations in the

French study (1). Then it was necessary to test varieties in sufficient locations to take into account this variability. The ranking of varieties can be very different in some locations (especially in sites with a drought stress before flowering time). To

evaluate varieties each year for seed protein content in the French network for variety registration, it has been found that 12 study locations can be reduced to six.

Protein content not related to yield or standing ability

It appears that there is no relationship between yield and protein content. When the yield is low, it is possible to have low protein content if the nitrogen nutrition is deficient at flowering time and after. That was the case in some areas of France in 2003. In contrast, if the nitrogen nutrition is good at flowering but the seed number is very low, the yield can be low and the protein content can be very high. This was the case in the north of France in 2005. It is also possible to have a high yield and a high protein content at the same time. This was the case in the north of France in 1999 when the climate was very favourable with regular rainfall in May and June, during seed setting and seed filling. In this particular vear, vields of 8 t/ha were recorded with protein contents of 25% or 26% for some varieties. It is therefore possible to combine high yield and high protein content within the limits of the theoretical relationship that links the seed composition with optimal yield (see Munier-Jolain and Salon, page 12). In legume plants, genetic improvement both of yield and protein content seems yet possible.

In the European Field Pea Network, with the exception of cv. Nitouche, the varieties with high resistance to lodging had low protein content. They were varieties bred in Germany or Denmark and registered in Europe. In contrast, the varieties with high protein content had low standing ability. They came from France, where there is a threshold for the protein content at registration. Now, the varieties registered in France have better standing ability and varieties registered in the European catalogue may have higher protein content than Eiffel or Swing. Therefore it appears that there is no relationship between protein content and standing ability (see insert).

Insert

Protein content variability in some European pea cultivars registered in 2000–2005

by Gaëtan Dubois*

Within the framework of the GL-Pro network, a grain legumes cultivar database (available soon at www.grainlegumes.com) has been developed to compile information on varieties registered in Europe, in particular data on physiological characteristics, market share and regional agronomic recommendations.

For 164 spring pea cultivars registered, the protein content varied from -1 to+3 points either side of the value for the control variety Hardy (Figure 1). The variation in protein content is about 4% with a median protein content around 23.5% of dry matter.

Figure 2 shows clearly that there is no relationship between the protein content and the crop height at harvest (i.e. the standing ability) for the most recently registered spring pea cultivars. When the reference varieties are considered, it appears that for some varieties crop height at harvest might be inversely proportional to the protein content. Other varieties show simultaneously a high protein content and a better standing ability than the control variety Hardy.

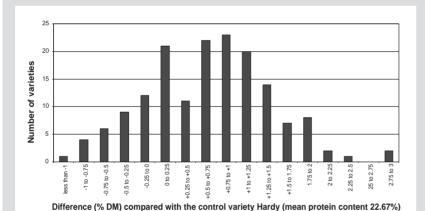


Figure 1. Protein content of some pea cultivars registered in Denmark, France and Germany in 2000–2005 compared with a control variety Hardy.

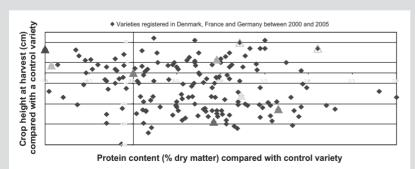


Figure 2. Protein content related to the crop height at harvest of some pea cultivars registered in Denmark, France and Germany in 2000–2005 compared with a control variety, Hardy. Each black diamond (◆) represents the data for a pea variety averaged over several years in one or more national network trials. The large triangles (▲) represent the data for some reference varieties considered as control cultivars with Hardy located on the vertical line.

Data sources: GL-Pro network —from national official trials for cultivar registration (DAAS-Denmark, BSA-Germany, CTPS & ARVALIS-France). *UNIP, Paris, France. Email: g.dubois@prolea.com

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Relating the performance of legume and other crops to the carbon costs of seed production¹

Relier performance des légumineuses et des autres cultures et coût en carbone de la production des graines

by Nathalie MUNIER-JOLAIN* and Christophe SALON*

ncreased crop productivity during the last 50 years is mainly a result of the Llonger duration of photosynthetic activity. The fate of assimilated carbon during the uptake and translocation of nutrients and photo-assimilates to the seeds has received much less attention because of the complexity of the underlying physiological mechanisms. However, for plant breeding, and in order to improve farmers' incomes and crop utilisation by industrial end-users, the need to increase yield without adversely affecting seed quality is a key challenge (http://www.eugrainlegumes.org). For legumes, the challenge has an economic implication because of the inverse relationship between high yields and high seed protein concentration. Furthermore, since 1960 the greater breeding effort for wheat compared with pea, as well as the poor overall efficiency of carbon and nutrient allocation to the harvested organs of pea are possible reasons for the higher annual yield increase observed for wheat compared with pea. In order to contribute to this debate, the relationships between yield and seed composition for different crops, or for different seed composition within a given crop, were related to theoretical estimates of the carbon cost associated with seed production.

Carbon costs associated with seed production: background

The carbon cost of metabolic processes associated with seed production is considerable. There is i) the carbon cost

*INRA - Unité de Génétique et d'Ecophysiologie des Légumineuses. Dijon, France. Email: munierjo@dijon.inra.fr, salon@dijon.inra.fr associated with the synthesis within seeds of the seed storage products (proteins, starch, lipids), ii) the carbon cost associated with the uptake of mineral nutrients, and iii) the carbon cost associated with nutrient translocation from the site of synthesis or assimilation to the sinks, including the seeds. According to Penning de Vries *et al.* (3) 'construction costs' can be defined as the amount of glucose required by the plant to build one gram of biomass, starting from minerals and glucose. This approach takes into account the metabolic pathways for the synthesis of the main compounds that

constitute plant biomass, expressed as the amount of glucose that is required to produce these compounds (3, 6). This value called Production Value Inverse (PVI) was calculated taking into account the needs of C-skeletons for energetic molecules (ATP) and for reducing equivalents (NAD(P)H). According to the metabolic pathway involved, PVI was found to be highly variable among the six categories compounds constitute most of the plant/organs biomass (Table 1).

• For a given crop, the mean seed composition (data available in feed composition tables (5)) and the PVI (the carbon cost associated with the synthesis of various storage

compounds) were used to estimate the carbon cost associated with the production of 1 g seed (Table 1).

• In the calculations of the carbon cost associated with the uptake of mineral nutrients, the total amount of soil nitrate taken up was considered to have been reduced within the roots, irrespective of the species. This implies that the carbon cost associated with nitrate uptake and reduction to NH₃ is identical between species. The assumption probably minimises the carbon cost associated with symbiotic nitrogen fixation and so reduces the

Table 1. Carbon cost (g glucose equivalent) required to synthesise 1 g pea seed.¹

Compound	PVI	Content	Energy costs				
		Pea	Pea				
	g glucose g ^{.1} compound	% of dry weight	g of glucose equivalent for producing 1 g of seed				
Cell wall	1.21 ^a	14.81 ^b	0.179 ^c				
Lignin	2.12	0.35	0.007				
Starch	1.21	51.62	0.625				
Soluble sugars	1.21	4.51	0.055				
Proteins	1.79	24.0 ^d	0.430				
0il	3.03	1.16	0.035				
Calculation							
A = Cost of syr	nthesis = sum of	the energy costs	1.331				
B = Cost of N	uptake and assin	nilation					
= $(d \times 2.5 \times 4.5)/(100 \times 6.5)$ 0.431							
C = Cost of tra	nslocation = A x	5%	0.067				
Total carbon cost	- N + B + C	1.829					

 1 Carbon cost associated with i) the synthesis of the various seed compounds, ii) nitrogen uptake and reduction to produce seed protein stored and iii) translocation of reduced nitrogen compounds are indicated and calculated as stated in the text. Carbon cost has been calculated as the product of PVI and seed percentage composition (c = a b). The cost of synthesis (A) is the sum of the various carbon costs for synthesising the various compounds. The cost of N uptake and assimilation (B), calculated in glucose equivalents (1 g C equals 2.5 g glucose), is the product of the protein content of the seed (% of dry weight) and the carbon cost of nitrogen assimilation (4.5 g C g 1 N), divided by the coefficient factor of the N content versus protein content of the seeds (6.5). The cost of translocation (C) is about 5% of the cost of synthesis.

difference between nitrogen fixing and non-fixing species. In our calculation an average value of 4.5 g C per g N (6) has been used as an estimate of the carbon cost of nitrogen assimilation.

• Absorbed nitrogen is reduced, and then translocated to sink organs in the form of amino compounds. Vertregt and Penning de Vries proposed a carbon cost of translocation equal to 5% of PVI (6).

Increase yield but maintain seed quality

Estimates of the energy costs of seed production explained in Table 1 are useful parameters for relating the seed composition of different species to their productivity (Figure 1). Eighteen species were analysed and the data used in Figure 1 were taken from international web sites containing yield statistics (http://epp.eurostat.cec.eu.int; http://faostat.fao.org). Because highly variable abiotic and biotic constraints occur across cropping areas, our simulations used data for the potential yield of a given species under field conditions in a major producing country where high yields are recorded.

It appears that the higher the calculated energy cost for producing 1 g of seed, the lower the mean yield: cereals, which are starch-rich, display the highest yields while oleaginous species have the lowest yields. Intermediate yields are obtained when seeds have a high protein content. For a given energy cost of seed production, the mean yield varies widely among species. Taking into account their seed composition, species with similar crop production performance

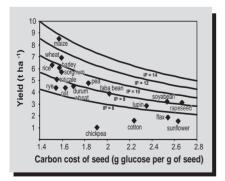


Figure 1. Relationship between the carbon cost of seed production for various species (5) and their yield (http://epp.eurostat.cec.eu.int, http://faostat.fao.org). Crop production performance is shown by IsoProduction (IP) curves, which express the product of energy cost per 1 g seed and yield.

(product of the energy cost of 1 g of seed and the yield) have their yield located on the same isoproduction (IP) curve (Figure 1). For crops such as maize or wheat, which have been improved significantly as a result of breeding, the harvest index and the yield are considered to be close to their maximum (4). As a consequence these species are located on the highest IP curves. Yield differences are still observed among plants sharing similar 'efficient' photosynthetic pathways (C4 plants) and having been improved in breeding programmes (for example, maize and sorghum). Within a given crop category such as cereals, proteaginous or oleaginous crops, those having similar energy costs for seed production may display widely different yields and be located on different isoproduction curves.

One hypothesis to explain these differences among species, is that they might result from the degree of genetic improvement through breeding, which has not been the case for lesser cultivated and/or profitable species. This hypothesis is confirmed by the observation that the species can be sorted in two main groups:

1) species with a high economic value, distributed over a large production area, and for which plant breeding has been very active and 2) less cultivated or subsistence species for which breeding programmes are still needed.

This observation suggests that for crops not yet improved by breeding, there is potential for increasing yields via selection without diminishing seed quality.

Energy costs higher for pea than wheat

The energy cost associated with the production of 1 g seed is 15% higher for pea than for wheat (1.83 and 1.59 g glucose-equivalents respectively). This 15% energy cost difference is mainly due to nitrogen uptake and assimilation and is probably an underestimate since the additional carbon cost due to symbiotic nitrogen fixation has been taken as minimal (see above).

In France, in intensive agriculture, the mean yield of field grown peas is about 30% lower than the mean yield of wheat. As such, under average agronomic conditions in Europe, our calculations suggest that the difference in seed protein content between

wheat and pea could explain, besides other physiological factors, a large part (about 50%) of the yield difference between these species.

Increase pea yield and protein content simultaneously?

This achievement could have an important economic impact because pea with a higher seed protein concentration would be desirable for poultry feed. The theoretical impact of a 10% increase in pea seed protein content on yield was calculated using the same approach as above. Taking into account the negative correlation between protein and starch concentration observed in many pea genotypes (1) the simulated increase in seed protein content in our calculations was compensated for by a reduction in the starch concentration of the seed: our calculation predicted a 3% theoretical reduction in yield, and the 10% increase in the carbon cost of nitrogen assimilation accounted for about 75% of the reduction (2). This yield reduction is probably underestimated because the real carbon cost of symbiotic fixation needs to be taken into account. Therefore, increasing the seed protein content by 10% would be of no benefit for farmers if the market price of pea increased by only about 2%-4%. However these calculations consider that yield potential has been reached and so represent the impact of an increase in seed protein content, when all others factors remain equal.

It remains possible that some genetic improvements through breeding may allow a simultaneous increase in yield and seed protein content as shown in the comparison of species.

¹This article is a shortened version of (2).

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Improvement of seed protein composition and quantity in grain legumes

Améliorer composition et quantité des protéines des graines de légumineuses

by Claire DOMONEY* and Mark STITT**

egume seeds are valuable sources of protein, starch and oil, which vary in proportion and importance, depending on the species. European grain legumes are grown primarily as protein sources for feed, and improvements in protein quantity and quality are highly desirable for the animal and poultry feed market. A better understanding of the fundamental aspects of assimilate uptake, transport, partitioning and metabolism in seeds, and the genetic factors that regulate all of these processes will facilitate the successful breeding or engineering of improved legume seed quality.

Measuring protein

The variation that exists for protein quantity among genotypes and environments is reported in the accompanying article (1). The values generally reported for protein content in crops are based on a conversion of determined nitrogen values and must be considered as relative rather than absolute values, since the conversion does not usually reflect the true ratio of nitrogen: protein found in plants. A conversion value of 6.25, valid for animal proteins, is commonly used, although the corresponding values for pea and wheat seed proteins have been determined to be 5.44 and 5.33, respectively (11). Thus the true protein content of these plants is lower than is claimed to be the case. In laboratory experiments, protein contents of seed extracts are measured by comparison with protein standards, usually bovine serum albumin or immunoglobulin, standards that are inappropriate for absolute quantification.

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In rare cases, amounts of individual proteins are measured immunologically, using specific antibodies and reference to a purified standard of the same protein (16); whilst this approach is specific and accurate, there can be problems associated with specificity, in that related proteins may not be recognised efficiently. For high-throughput work, there has been a move towards the use of near-infra-red techniques for total protein determination (2, 15), whereas recommended standardised procedures are being developed for total nitrogen and crude protein content determination in cereals, pulses, milled cereal products, oilseeds, oilseed residues and animal feeding stuffs (ref. ISO 16634: Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content).

Clearly, elevating the content of protein in grain legume seeds must include considerations of protein quality, and any increase in protein content should be accompanied by a corresponding increase in digestible protein and amino acid absorption by animals. Figure 1 shows the elevated protein content of seeds from plants that have been grown under drought-stress

conditions; an increase of approximately 5% dry matter was observed. As yet, the nature of the protein increase in this study or in related work (6) has not been determined; the latter work suggests that temperature affects the activities of some gene promoters. An elevated amount of certain proteins is undesirable. For example, seeds contain inhibitor proteins that interfere positively with digestion, as well as proteins that are relatively resistant to digestion and can lead to an immune response (4, 17, 13). The major quantitative trait locus responsible for variation in trypsin/chymotrypsin inhibitor content has been identified, molecular markers are available for breeders (12), and the benefits of a low content of these proteins to young broilers has been demonstrated clearly (19). A second albumin protein, PA2, is poorly digested and, in chickpea, is linked to allergic responses. Within GLIP (www.eugrainlegumes.org), quantitative variants in PA2 content are being studied to determine the basis of pleiotropic effects associated with this variation. Preliminary metabolite profiling experiments on developing seeds show differences in metabolites between these variants and also between lines having

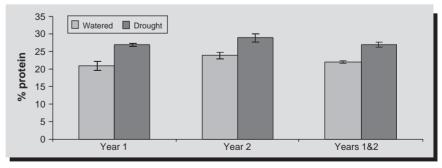


Figure 1. Protein content of seeds from plants grown under controlled watered or drought-stress conditions over two growing seasons. (Courtesy of Catherine Chinoy, JIC).

different overall protein content. A major challenge is to unravel the effects of specific loci, through the analysis of selected recombinant inbred and back-cross lines. For many of these genes/loci, such analyses must be performed in legumes, as there are no apparent homologues in Arabidopsis. Seeds of Medicago truncatula store a higher proportion of protein than either pea or faba bean and, within a sample of 50 ecotypes or cultivars of diverse geographical origin, seed protein content varied between 33 and 46% (10). As such, this species offers potential to dissect the overall control of partitioning that leads to a higher seed protein synthesis, and may facilitate the precise genetic mapping of controlling loci.

The genetic basis for quantitative variation in individual seed proteins has been described in some cases; for example, mutations affecting the active site of pea trypsin inhibitor proteins (12), structural genes and quantitative trait loci affecting the accumulation of PA2 (GLIP, Domoney et al., unpublished), and lipoxygenase (8), are evident. The exploitation of such mutants depends on their impact on physiological functions. Many genes that are active during seed development may have additional functions in other aspects of vegetative plant growth, for example, lectin genes and root nodulation; in other cases, related but distinct genes are differentially regulated, for example the trypsin inhibitor genes that are expressed in seeds, roots and flowers (7) and the closely related PA2 and END 1 genes in seeds and flowers, respectively (9).

Loci affecting seed shape are important

A major effect on seed protein content in pea is exerted by loci that affect the shape of the seed dramatically; these loci have been studied in a group of *rugosus* (*r*) or wrinkled-seeded mutants, where changes in the proportion of protein, starch and lipid are a consequence of lesions in starch biosynthesis (e.g. a mutation in starch branching enzyme, *sbe1*, in the case of the original Mendel character, *r*) (18). A higher protein content in the *r* mutant is associated with a decreased legumin synthesis (16), an effect that is opposite to that reported for some environmental variation (1). As the fine map positions of quantitative trait

loci influencing seed composition become available (see below), linkage with further loci influencing seed development and morphology may become apparent. A wrinkled-seeded mutation in *Arabidopsis* is unrelated at the genomic level to those of pea, and is caused by a splicing mutation in a transcription factor (3). In this context, it is interesting that a large number of genes are currently being identified in *Arabidopsis* that modulate starch levels and content. This may provide several candidates for assessment in seed protein control, using marker technology or eco-tilling (14).

Unravelling control of seed composition

Quantitative trait loci (QTL) affecting total seed protein accumulation in pea are being mapped in a number of laboratories and, within GLIP, lines with elevated protein content are being subjected to metabolomic and genomic analyses to unravel the molecular basis for genetic differences. Between three (15) and seven (2) loci have been reported to contribute to variation in seed nitrogen content in crosses between round-seeded pea lines; in the latter work, these include le (internode length) with a large effect. Four linkage groups of Tar'an et al. (15) have not been assigned as yet to the consensus pea genetic map. Genomic sequencing projects will assist with identifying candidate genes that are associated with the protein/nitrogen QTL. In Arabidopsis, transcription factors (TFs) that are preferentially expressed during seed development, compared with leaves, are being defined by real-time RT-PCR (5) and the functions of these are being unravelled by RNAi. Ongoing examination of the relative expression of TFs during embryogenesis in Medicago, together with mutational analysis, will identify TFs that are 'master controllers' of seed composition.

Advances in plant genomics may also open up further strategies to improve seed protein levels. A central question is whether protein levels are dependent on the levels of expression of storage protein genes and/or genes that allow these rather special proteins to be processed and stored, or if storage protein levels are dependent on the rates of nitrogen uptake and assimilation and the rates of nitrogen reallocation to filling seeds. Analyses of changes in metabolism and

metabolite levels within the vegetative plant organs and the developing seed, in parallel with analyses of seed protein levels, could provide an answer to these questions. Most importantly, this kind of comparative physiological approach was hampered previously because it is essentially descriptive in nature. New perspectives are opened by the possibility of combining detailed phenotyping with modern tools for genotyping plants. Such analyses, when conducted across large sets of cultivars and in appropriate recombinant inbred or nearisogenic lines, could allow the dissection of a complex trait like 'high protein'. This will lead to the identification of a series of QTL, each of which is associated with a defined molecular or physiological phenotype.

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The relationship between protein content and protein composition of pea seeds

Relation entre la teneur et la composition protéique des graines de pois

by Judith BURSTIN* and Gérard DUC

aw materials with a high and reliable protein nutritional value are crucial for feed manufacturers. The protein value of pea seed has different components relating to both the content and the composition of protein. High protein content allows higher rates of incorporation in pig or poultry diets, ensuring that peas are a good complement to cereals in feed rations. Composition relates to the different storage protein fractions. The three major protein fractions in pea seeds are the 11S legumins – compact hexamers of 350 to 400 kDa associated with acidic alpha-polypeptides and basic beta-polypeptides, the 7S vicilins and convicilins - trimers of 150 to 280 kDa composed of very heterogeneous and differently processed polypeptides, and the 2S albumins containing very diverse proteins, among which the 11 kDa PA1 and 48 to 53 kDa PA2 are considered important storage fractions. These fractions do not have equivalent in vitro and in vivo digestibilities (5, 4), and their amino-acid composition is different, particularly with respect to sulphur-rich amino acids which are rare in vicilins but high in albumins PA1 and PA2 (3).

Several authors have reported a large variation in crude protein content as well as in the protein composition of pea seeds. For example, surveys conducted by UNIP in France revealed a large range of protein content in seeds collected on farms, reinforcing the need for monitoring and stabilising pea seed protein value so that

*INRA URLEG, Domaine d'Epoisses, 21110 Bretenières, France. Email: burstin@epoisses.inra.fr users have more confidence in the product. In pea, a method based on ion-exchange chromatography (FPLC) set up at INRA-Nantes (1) was used to investigate seed protein composition for different genotypes and environments. These studies revealed both genotype and environment variability. In this article, in order to propose strategies to stabilise the nutritional value, we will give some examples of the genotypic and environmental variability of pea seed protein content and composition, and examine how they relate to each other.

Environment has a large effect

For a given pea genotype grown in different environments, a large range of variation in crude protein content was observed frequently. For the cultivar Amino, Gueguen and Barbot (7) found a crude protein content varying from 18.1% to 27.8% of seed dry matter. This variation was associated with a modification of protein composition, the lower content being associated with a doubling of the vicilin to legumin ratio. Figure 1 gives another example of the variability in protein composition of seeds of a single pea line grown at two locations in France. Again, high protein content was associated with a high legumin peak as revealed by the FPLC profile (Cassecuelle and Duc, pers. com.). Numerous authors have reported that pea seed protein level is markedly affected by environmental variables (8, 9). Among these variables, factors affecting the efficiency of plant nitrogen nutrition are important, as illustrated by Duc et al. (6) in an experiment with a non N-fixing pea line grown in the presence or absence

of nitrogen fertiliser. The resulting seed protein content (DM basis) was 26.3% with nitrogen fertilisation, and 16.9% without fertilisation and the low protein content was associated with a doubling of the vicilin:legumin ratio. In these examples, the variation in composition is a quantitative variation, mainly expressed as a variation in the height of the legumin peak on FPLC profiles.

Genetic variability is available

Within the framework of the EU ECLAIR project, a survey of the variation in seed composition was carried out on 54 pea genotypes grown in the same environment (2). A large range of genetic variability for seed crude protein content was identified: from 20.1% to 28.1% in feed peas, from 21.0% to 26.9% in colouredseeded peas and from 25.6% to 29.1% in wrinkled-seeded peas. Within the feed pea group, the proportions of the different protein fractions, estimated by integrating FPLC peak areas, varied with genotype: 33-57% for albumins, 24-45% for vicilins and 17-39% for legumins. In another experiment, we analysed the protein composition of the seeds of 120 pea genotypes grown in one environment, and found a large genetic variability among both smooth and wrinkled seeds (Figure 2, A and B). The FPLC method distinguished two types of variability, a qualitative one with peaks appearing at different retention times, and a quantitative one with different heights of corresponding peaks. Although there was no clear link between seed protein content and seed composition among wrinkled seeds, there

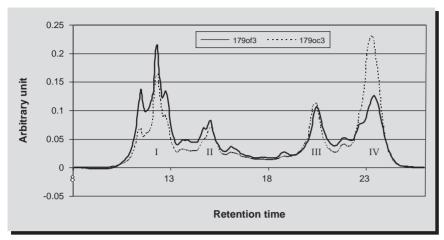


Figure 1. FPLC protein fraction profile for a pea breeding line 179 grown at two locations, of 3 and oc 3. The fraction I corresponds to albumin PA2, II to albumin PA1, III to vicilin, and IV to legumin. The height of the different peaks cannot be compared for the different fractions but can be compared for a same peak for different genotypes and or environment.

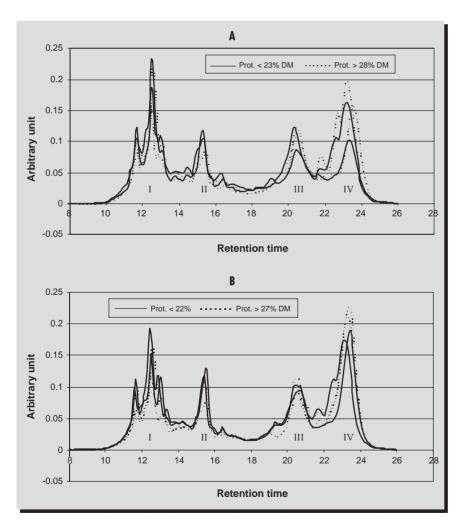


Figure 2. FPLC protein fraction profiles for genotypes with contrasted protein content. A. Wrinkled-seeded genotypes with protein content below 23% DM and above 28% DM. B. Round-seeded genotypes with protein content below 22% DM and above 27% DM. The fraction I corresponds to albumin PA2, II to albumin PA1, III to vicilin, and IV to legumin.

was a trend among smooth seeds for an increase in the globulin:albumin ratio in protein-rich seeds.

Interactions will require a better understanding

Numerous studies have demonstrated significant genetic variability in seed protein composition and this offers the possibility of breeding for improved protein nutritional value. For both sources of variation, environment and genotype, high protein content was often associated with increased legumin content in round-seeded peas. This variation in legumin content (legumin peak height measured by FPLC) could be the expression of plasticity in the response to varying levels of nutrient supply to the seeds. To breed for improved and more stable protein nutritional value, the link between protein content, protein composition, and nutrient supply to the seeds will need to be assessed further. This will require a better understanding of the genotype x environment interactions for these traits, and an identification of the factors of instability - drought stress, high temperatures and symbiosis mechanisms.

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Genetically increasing seed protein content and quality in faba bean

Amélioration génétique de la teneur en protéine et de la qualité des grains de fève

by Wolfgang LINK*, Hans WEBER** and Gérard Duc***

efore the twentieth century there was a long tradition of faba bean (Vicia faba) cultivation in Europe for food (dry or fresh seed) or animal feed (dry seed or forage). The Marshall Plan in 1946 resulted in a reduction of European grain legume production; the soyabean crisis in 1973 resulted in strong EU encouragement for faba bean by regulations and support for research; mad cow disease in 1996 and more recently since 2000 the Aphanomyces disease on pea have contributed to a significant boost in faba bean production in Europe, increasing from 0.6 Mt in 1997/98 to 1.3 Mt in 2004/05. In parallel, the increasing concern about food safety and sustainable and organic agriculture has reinforced the interest in faba beans.

Faba bean seeds contain about 40% starch and 30% protein and a low (1%-2%) fat content. The ECLAIR-EU action (3) detected a range of 26.1% to 38.0% protein in the seed DM in a collection of types containing tannins. This is in agreement with other explorations of genetic variability. High heritability estimates promise marked improvements from breeding for this trait (5, 10). In the ECLAIR-EU genotypes, a strong negative correlation (r = -0.67, 72 d.f.) was found between seed protein and starch contents. No effect of the vc-gene (which reduces the vicine and convicine content of seeds) on protein content was detected. Zero

The amino acid composition of the seed is determined primarily by its protein content (3). Compared with soyabean or cereals, faba bean seeds are rich in lysine and poor in methionine and cysteine. In the European DG VI CAMAR project (1995), the lysine content of genotypes with seed protein levels higher than 30% was 5.7 to 6.5 g/16g N, whereas the range for methionine + cysteine was only 1.7 to 2.3 g/16g N. Frauen (4) in a survey of n = 287 genotypes found a strong negative genotypic correlation between methionine per protein and protein per seed dry matter (r = -0.71), and Sjödin et al. (11) detected unfavourable correlations between total protein and protein fraction composition (see below). Obviously, a straight selection for more protein and more methionine per protein is not very promising, so a more detailed understanding is necessary.

Detailed structure of storage proteins

Faba bean seed proteins contain 15–20% albumins and 80–85% globulins (7). Other proteins like leginsulin (8) are considered to play a role in insect defence. Globulin contains about 25% vicilin and 75% legumin (7). Both proteins occur in many species including *Medicago truncatula* and *Lotus japonicus*.

The sulphur content of vicilin is extremely low. The DNA that codes for

the more prevalent type of vicilin subunits shows no methionine and cysteine codons. Legumin is considered to be a protein with higher nutritional value. The ratio of legumin to vicilin among genotypes varied between 1:2 and 1:3.7 (7). Biosynthesis and seed deposition of vicilin start several days earlier than for legumin (1). The biosynthesis of legumin continues until later stages of seed development than the biosynthesis of vicilin, so stress at later stages will decrease the legumin content of seeds. The ratio of vicilin to legumin is much higher in the embryonic axes than in the cotyledons (7). Perhaps seed size and shape influence the ratio of cotyledons to embryo axis and thus the legumin to vicilin ratio, and eventually the sulphur content of the protein.

The relative molecular mass (*M*) of legumin approaches about 330,000 – 350,000. Vicilin is a smaller protein (*M* about 150,000). Legumin is made up of six subunits (Figure 1). Every subunit is composed of one alpha- and one betachain, which are connected by disulfide

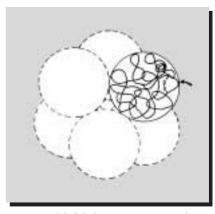


Figure 1. Model of the hexamerous structure of legumin (based on (9).

tannin material tended to have higher protein contents than tannin-containing types, partly due to the reduction of seed coat proportion in these types. The trypsin inhibitor activity in the faba bean seed protein is low (0.3 to 5.3 units/mg DM (3)) in comparison with many other legumes.

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Seed of faba bean (Vicia faba).

bonds. The subunits are a direct gene product, one alpha and its beta chain being transcribed as one common mRNA. Thus, variation in legumin subunits is a direct reflection of allelic variation (13). Probably the legumin molecule is composed more or less at random of the genetically different subunits that are available (7).

There are two main types of legumin subunits: legumin type A and legumin type B, both having an M of about 60,000. In the A legumin subunit, both the alpha- and the beta-chain contain methionine (as well as cysteine). Type B legumin subunits do not contain methionine. Approximately 50% of the faba bean legumin subunits are devoid of methionine. Two of the several known versions of type A legumin are very similar to each other, but the amino acid sequence of type A legumin and type B legumin are markedly different (7). The members of the legumin and vicilin gene families are located as clusters on the chromosomes. The legumin type B3 and B4 genes are, for example, located at a distance of 7.2 cM from each other on chromosome 3 (12).

The number of legumin A genes is expected to be less than 10 and the number of legumin B genes is about 12. DNA clones for two type A legumin genes are available, and as well for four type B legumins (6).



Faba bean flower.

Tucci *et al.* (13) demonstrated by SDS-PAGE the diversity of band patterns from total seed proteins. Two distinct alpha chains of legumin with M=35,100 and M=34,300 were proven to follow a simple Mendelian segregation. These data show that probably legumin SDS bands could be used as markers in an association mapping approach to test and eventually use their influence on seed protein characteristics for breeding.

Analysis of seed protein metabolism

An approach for a deeper understanding of the genetics of the trait is the analysis of pertinent enzymes. At the IPK Gatersleben transgenic Vicia narbonensis and Pisum sativum with altered availability of C and N to the seed (15) are used. Seed specific expression of an amino acid permease from Vicia faba (VfAAP1; it catalyses amino acid uptake) in pea and V. narbonensis increased seed nitrogen content by 10% to 25%. It was mainly the synthesis of vicilins and legumins rather than albumins that was stimulated. ¹⁵N uptake experiments indicated increased sink strength for amino acids. Increased seed size and vegetative biomass of transgenic plants suggested a stimulation of their growth rate and duration probably due to an improved N status. Transgenic V. narbonensis seeds expressing a bacterial PEP carboxylase also accumulated 20% more protein and had higher seed weight. Physiological and biochemical analyses indicated a shift of metabolic fluxes from sugars and starch to organic acids and free amino acids. The conclusion is that seed protein synthesis is limited by the supply of amino acids and by carbon acceptor availability. Thus, increasing seed sink strength should stimulate seed protein synthesis and growth.

As a further step towards understanding gene regulation, Dimitri *et al.* (2) also using *V. narbonensis*, combined the transfer of a foreign gene for a methionine-rich protein (Brazil nut albumin) with the expression of a bacterial aspartate kinase that is known to stimulate methionine biosynthesis. There was a 10% to 12% increase of methionine in mature seeds of the aspartate kinase transformants and an 80% increase in the Brazil nut 2S albumin transformants. Double

transformants showed additive effects of the two foreign genes on seed methionine content. In their mature seeds proteinbound methionine reached levels 2.0 to 2.4 times higher than in the wild type.

For faba bean the wealth of data summarised here shows that although upgrading faba bean diversity by genetic transformation is neither readily available nor publicly accepted, its natural allelic diversity should allow a marked improvement in its protein content and quality. The prerequisite is a detailed knowledge of its genetic foundation and a very tight cooperation between model crop research and applied crop genetics.

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Economic value of pea protein in feed

Valeur économique de la protéine du pois en alimentation animale

Katell Crépon* and Frédéric Pressenda**

n Europe peas are used mainly for pig nutrition, where their nutritional value If its perfectly with pigs' nutritional requirements. Of the 870,000 tonnes of peas used during 2004–2005 in the French feed industry, about 90% were used for pig formulas (Table 1, standard pea), of which half was for finishing pig formulas. In these types of formula, from 50% to 80% of the cost of the feed is attributed to the energy constraint, that is to say that the value of pea is mainly enhanced by its energy content. In other words, its shadow price¹ is due at present mainly to its energy value (see Insert 1). So, although pea is a raw material rich in protein, its price is determined mainly by its energy content. In poultry formulas, the shadow price of pea is decreased by a strong 'concentration' constraint (expressed by the last figure in bold in the formula indicated in Insert 1). This constraint expresses the necessary concentration in nutrients of raw materials used for poultry nutrition.

Indeed, if the mixed profile of pea (supply of protein and energy) is a strength for pig nutrition, its low nutrient concentration is a weakness for poultry nutrition where the main sources of protein used (soyabean meals) are very concentrated.

Therefore, one way to gain a greater share of the poultry market could be to increase the protein content of pea. Two strategies can be implemented:

 the first consists of using the natural variability in protein content found in cultivated peas, sorting out the grain at harvest, and putting on the market two types of pea according to their protein content,

Table 1. Potential uses of peas with different characteristics (year 2004/2005).

1000 t	Rumi	nants	Pi	gs	Pou	ltry	Otl	ners	Total
Standard pea	36	4%	774	89%	39	4%	23	3%	871
Pea highly digestible*	50	6%	570	70%	148	18%	4	6%	813
Pea 28% CP**	22	3%	461	58%	283	35%	31	4%	797
Pea 28% CP Energy value → -1%	22	3%	430	60%	254	33%	30	4%	735
Pea 28% CP Energy value 3 −5%	14	3%	324	70%	99	21%	23	6%	461
Pea 28% CP Energy value → −10%	6	2%	195	80%	21	9%	21	9%	243
* Price +€16/t compared with standard pea; ** Price +€7/t compared with standard pea; CP =crude protein.									

 the second is based on breeding peas with higher protein contents or more digestible amino acids.

Pea protein content exhibits natural variability

Expressed on a dry matter basis, the average level of protein for pea is 24%, with a standard deviation of 1.4². The variability is usually considered as a constraint by feed manufacturers because of its consequence on the variability of the feed nutritional content. Nevertheless, this variability could also be used by stockpiling agencies to select different qualities of peas. The potential of a market segmentation, according to the protein content of peas, has been studied using the model 'Prospective Aliment', which is able to simulate the feedstuffs supplying strategies of French feed

manufacturers. The sorting strategies that have been studied involved selecting 30% of the peas harvested with the highest protein level. The first category has an average protein content of 26% of DM, the remaining 70% has an average protein content of 23.7% of DM.

According to the model, the market segmentation leads to an increase in the volume of peas used by 5% to 8%, depending on the economic context, when the two types of peas are proposed at the same market price. The average price of formulas in the feed industry decreases by only 0.3t or 0.7t (depending on the economic context), which means that if the feed industry is able to use more pea when the market is segmented, it is not able to pay a lot for this segmentation.

Insert 1

Estimation of shadow prices of pea (SPpea) according to average nutritional constraints costs (2004/2005)

'n a finishing pig formula

SPpea $(\leqslant/t) = 45*NE(Mcal/kg)+0.2*CP(\%)+1.5*LysDig(g/kg)+1.6*MetDig(g/kg)+3*thrDig(g/kg)+4*TryDig(g/kg)-$ **14**In a finishing broiler formula :

NE : Net Energy value ME : Metabolisable Energy value CP : Crude Protein content

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If only 10% of the harvest – and not the whole market as seen previously – is segmented with the same strategy (30/70), the added-value may be estimated for the stockpiling agencies at about €1.5/tonne while the cost of such a segmentation has been estimated at €2.2/tonne. We can conclude that the natural variability of pea protein cannot make segmentation a lucrative market.

Increased protein digestibility or protein content?

It may also be possible to improve the protein content or the protein digestibility by breeding. Two hypotheses have been tested:

- 1. Breeders are able to produce a pea with an average protein content of 28% of DM (pea 28) without modifying its amino acid profile or its energy value
- 2. Breeders are able to produce a pea in which the digestibility of methionine, cystine and tryptophane is increased by 10 points (pea dig).

These 'new' peas have been introduced in the Prospective Aliment model. They vary from the standard pea only in the characteristics described above. The other raw materials and factors considered in the model remained unchanged (e.g. nutrients, prices). The results are based on the lowest cost optimisation method (linear programming) that is used by the feed manufacturers to determine their buying and formulation policy.

According to the Prospective Aliment model, the potential market for pea 28 in 2004/2005 would have been about 2 million tonnes, if its price market had been the same as that of standard pea. During the same period, the potential market for pea dig would have been 1.4 million tonnes.

To keep the outlet at about 800,000 tonnes, the potential added-value for pea 28 compared with the standard pea is €16/t (Figure 1) and the added value for pea dig is €7/t (Figure 2). Pea 28 seems to be the best strategy to penetrate the poultry market since it targets the high protein concentration required for pea used in poultry formulas (Table 1).

Maintain the energy value

All these simulations are based on an increase in protein, with no other effects

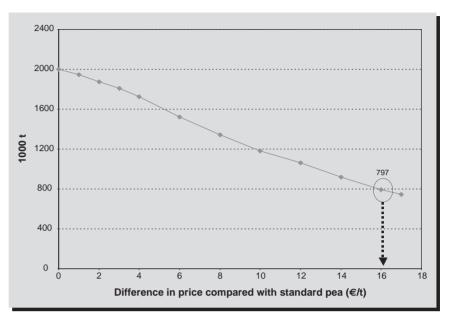


Figure 1. Evolution of the uses of pea enriched in protein according to its price compared with a standard pea.

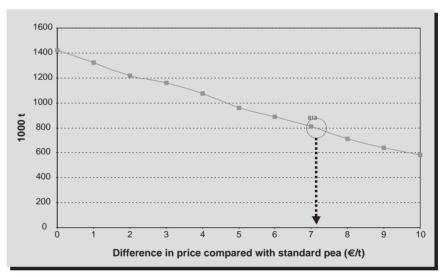


Figure 2. Evolution of the uses of a more digestible pea according to its price compared with a standard pea.

on the nutritional composition of pea. Yet it has been shown that the protein content is strongly linked with starch content. An increase in protein content will lead to a decrease in starch content, and could thereby have an impact on energy value. The model shows that a reduction of 1%, 5% or 10% of the pea energy value would lead to a decrease of, respectively, 7%, 42% and 69% of pea 28 uses (if the prices do not change). The breeding strategy that involves increasing amino acid digestibility would be more efficient for increasing pea uses since it is unlikely to lead to a decrease in energy content.

The conclusions are mixed. Increasing the protein content or the content of digestible amino acids in pea is obviously interesting and may extend the number of formulas able to incorporate peas. But the increase in protein content must be done without damaging the energy value. Even a slight decrease in energy may have a strong impact on the price and volume of pea used.

¹ Shadow price: the price below (above) which a raw material becomes economically interesting (not economically interesting) for being incorporated in an industrial compound feed formula.

² AFZ, Io-7, la banque de données de l'alimentation animale, 2005.

Protein crops in 2005: areas, yields and production in the EU

Les protéagineux dans l'UE en 2005 : surfaces, rendements et production

by Gaëtan DuBois* on behalf of GL-Pro partners and associated experts**

rain legumes can be regarded in different groups according to the European Agricultural Policy. Protein crops, which include field pea, faba bean and sweet lupins, represent more than 70%, mainly peas and faba beans, of the EU grain legume production and are classified as arable crops that benefit from a compensatory protein subsidy. Soya, which is classified as an arable oil crop, represents about 15% of EU grain legumes production and beans and vetches, considered as alternative crops (not part of the arable crops), each represent about 2.5% of EU production.

From a dry spring to a wet summer

Sowing conditions were generally good all over Europe, but low temperatures during February and March, with many frosts, caused losses of up to 10% in Spain. Some early sown plants were also damaged by frost at the end of April, especially in the East of Europe where temperatures were as low as –13 °C in East Germany and –9 °C in the south of the Czech Republic.

The main climatic event in 2005 was the drought. The winter of 2004 was rather dry, especially in Spain but also in other parts of the EU including France and East Germany. Rain did not occur in Spain before April and, as a result, affected all crops significantly. The exceptional and long heatwave at the end of June in

From July a long rainy period with flooding occurred so that in many regions, such as Germany and the Czech Republic where grain legumes are later maturing, harvesting was difficult or impossible because of the wet conditions and lodging problems.

Diseases played only a minor role in 2004/05. Pests attacks were lower than in 2003/04 but still damaging in some regions: black aphids in Germany, *Bruchus* on faba beans in France and Belgium and on peas in southern France and South Moravia, *Contarinaria* in eastern France and *Sitona* in the Czech Republic.

Bad weather for pea

In 2005, after three years of relative stability, the European area of field peas decreased by about 5.5% to 826,000 ha. Added to this area decrease, the low yields (about 3 t/ha on average at the European level: 13% lower than the average of 3.5 t/ha for the previous five years) had an adverse effect on pea production. Estimated production was around 2.5 million tonnes, more than 650,000 tonnes (20%) lower than in 2004 (Figure 1).

These general tendencies hide a large amount of variation among different areas of the EU (Table 1). In Spain, the pea area is still increasing (8% higher than in 2004). In Castilla y León, the area increase was

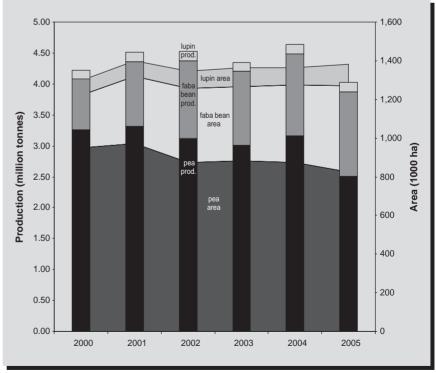


Figure 1. Area and production of peas, faba beans and lupins in the EU.

northern France affected this important grain legume production area.

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^{**}Overview compiled within the scope of the concerted action GL-Pro (European extension network for the development of grain legumes production in the EU) with the collaboration of the official partners of the project and associate experts: www.grainlegumes.com/gl-pro/

about 14% reaching 67,800 ha, which is almost half of the Spanish pea area. However, yields, as for all other crops, were affected badly by frost and drought. Pea yields were only 0.82 t/ha, which was 36% lower than the 2000–2004 average of 1.27 t/ha. Northern regions like Navarra and Aragon were relatively less affected with losses of about 5% and 17%, respectively.

Among the other major producers the pea area is still decreasing: France (-8%), Germany (-9%), United Kingdom (-15%), Austria (-8%), Sweden (-6%), Denmark (-40%). In the Czech Republic and Poland, the pea area increased compared with 2004 (+35% and +22%) returning to the levels in 2002 but still lower than in 2000 when the area in each of the countries reached about 35,000 ha.

With the exception of the northern countries where the yields are more stable (UK with 3.6 t/ha compared with 3.5 t/ha in 2004 and 3.58 t/ha for the 2000-2004 average, and Sweden with 3.2 t/ha compared with 3.3 t/ha in 2004 and 3.03 t/ha on average), EU vields in general were lower than in 2004. In Germany with yields of 3.2 t/ha in 2005, they were close to the 2000-2004 average of 3.15 t/ha, but 16% lower than in 2004 with 3.8 t/ha. In fact, higher yields of 4-5 t/ha were registered in the North (Schleswig-Holstein) and in the South (Bavaria) but lower yields of about 2.4 t/ha were registered in the continental regions of the North-East (Brandenburg, Mecklenburg-Vorpommern).

In France, which is still responsible for more than 50% of EU production, the national yield of 4.2 t/ha was 10% lower than the average for the five previous years. Actually, the yields in the South were similar or even better than in 2004, but in the northern regions, where the majority of protein crops are grown, yields were most adversely affected by the heatwave at the end of June. In fact, in Picardie, Champagne-Ardenne, Ile-de-France, Haute-Normandie, Nord-Pas-de-Calais which grow more than 50% of the French pea production and more than 25% of European pea production, yields were 14% less than the mean for 2000-2004. Winter peas, earlier than spring peas, were less affected by the heatwave. Their yields were often better, but they were sometimes difficult to harvest.

Faba bean is still expanding

The faba bean area is still expanding. In 2005 it increased to about 446,000 ha, an increase of 11% compared with 2004. The increase over the last five years is about 175,000 ha (+64%). The yields of 3 t/ha, were lower (-7%) than in 2004, but close to the 2000–2004 yield average (-2%). As a result of the expanding growing area, production has continued to increase. In 2005, with a production level of 1.365 million tonnes, it exceeded for the first time a level equivalent to half the European production of pea which still remains the most important grain legume produced in the EU (Figure 1 and Table 2).

Faba bean areas are increasing primarily in France (+30%), Spain, (+10%) and also

in the UK (+4%), which still produces more than half of the European production. In the UK, yields remained stable at 3.8 t/ha. For Germany and Italy, yields were lower than last year (-10% and -19%, respectively) but also close to the 2000–2004 average.

In Spain, almost 90% of the faba bean production is located in the South: Andalucia and Extremadura. The yields were affected adversely by frost and drought and were lower than 0.5 t/ha, 60% less than the average for 2000–2004.

In France, faba beans were later maturing and thanks to the cooler and wetter weather in July in the north, were able to recover some yield so that the final level was 4.13 t/ha and only 6% lower than the mean for the years 2000–2004.

Table 1. Area (1000 ha), yield (t/ha) and production (thousand tonnes) of peas in European countries.

		2005	Difference 05/04	Average 2000–04	Difference 05/00-04	Production 2005	
France	Area Yield	326.0 4.16	-7.9% -12.1%	377.6 4.62	-14% -10%	1,356	
Germany	Area Yield	111.1 3.21	-8.6% -15.9%	142.2 3.15	-22% 2%	357	
United Kingdom	Area Yield	53.0 3.6	-14.5% 2.9%	81.0 3.58	-35% 1%	191	
Spain	Area Yield	146.9 0.82	8.3% -43.2%	82.2 1.27	79% -36%	120	
Austria	Area Yield	36.0 2.50	-8.4% -19.5%	40.5 2.58	-11% -3%	90	
Sweden	Area Yield	25.0 3.2	-5.7% -3.6%	26.0 3.03	-4% 6%	80	
Czech Republic	Area Yield	29.0 2.7	34.9% -18.7%	28.1 2.52	3% 8%	79	
Denmark	Area Yield	16.0 3.35	-40.1% -6.9%	33.3 3.73	-52% -10%	54	
Poland	Area Yield	19.5 1.8	21.9% -26.2%	23.6 2.10	-17% -12%	36	
Italy	Area Yield	11.0 3.1	10.0% -3.4%	7.4 3.20	49% -3%	34	
Slovakia	Area Yield	12.0 2.5	9.1% -16.7%	12.4 2.02	-3% 24%	30	
Lithuania	Area Yield	12.5 1.6	8.7% -16.4%	16.9 2.04	-26% -22%	20	
Hungary	Area Yield	11.5 1.6	-47.7% -44.7%	23.6 2.16	-51% -26%	19	
Netherlands	Area Yield	2.5 4.4	-7.4% -15.1%	2.3 4.23	9% 4%	11	
Finland	Area Yield	4.0 2.3	33.3% 12.5%	4.5 2.26	-11% -1%	9	
Latvia	Area Yield	3.0 1.7	100.0% -7.4%	1.8 1.78	67% -7%	5	
Belgium	Area Yield	1.2 3.6	-29.4% -2.7%	1.5 4.20	-20% -14%	4	
EU	Area Yield	826.0 3.030	-5.4% -16.3%	909.6 3.49	-9% -13%	2,502	
Sources: UNIP, Paris, France — EUROSTAT and national databases.							

Table 2. Area (1000 ha), yield (t/ha) and production (1000 tonnes) of faba beans in European countries.

		2005	Difference 05/04	Average 2000–04	Difference 05/00-04	Production 2005
United Kingdom	Area Yield	188.0 3.8	4% 3%	161.20 3.770	17% 1%	714
France	Area Yield	105.0 3.9	30% -15%	63.38 4.130	66% -6%	410
Italy	Area Yield	45.0 1.5	2% -19%	49.40 1.537	-9% -2%	68
Germany	Area Yield	16.4 3.7	6% -10%	18.44 3.606	-11% 3%	61
Poland	Area Yield	12.0 2.5	20% -11%	14.60 2.357	-18% 6%	30
Spain	Area Yield	53.1 0.5	10% -62%	31.26 1.163	70% -60%	25
Austria	Area Yield	3.5 2.9	17% 7%	3.20 2.613	9% 9%	10
Sweden	Area Yield	5.0 2.0	-9% -8%	3.50 2.026	43% -1%	10
Czech Republic	Area Yield	3.0 2.5	-54% 14%	4.20 2.040	-29% 23%	8
Lithuania	Area Yield	4.0 2.1	70% -11%	2.18 1.969	79% 4%	8
Greece	Area Yield	2.3 1.7	0% -11%	2.56 1.759	-10% -1%	4
Belgium	Area Yield	0.7 3.9	75% -22%	0.38 4.220	84% -9%	3
EU	Area Yield	446.0 3.1	11% -7%	358.13 3.140	25% -2%	1,365

Table 3. Area (1000 ha), yield (t/ha) and production (1000 tonnes) of lupins in European countries.

		2005	Difference 05/04	Average 2000–04	Difference 05/00-04	Production 2005
Germany	Area Yield	38.2 1.5	7% – 25%	35.7 2.20	7% -32%	57
Poland	Area Yield	28.9 1.3	149% -20%	11.1 1.35	159% -3%	38
France	Area Yield	8.5 2.4	-11% -11%	12.3 2.50	-31% -4%	20
United Kingdom	Area Yield	7.0 2.6	0% -5%	3.3 2.67	115% -3%	18
Spain	Area Yield	13.9 0.45	-12% -30%	14.2 0.71	-2% -36%	6
Italy	Area Yield	4.0 1.3	0% 0%	4.0 1.38	0% -6%	5
Lithuania	Area Yield	5.0 1.00	92% 7%	1.9 1.08	160% -7%	5
Portugal	Area Yield	2.0 0.8	0% 0%	2.5 0.79	-20% 1%	2
EU	Area Yield	107.9 1.41	22% -20%	85.7 1.76	26% -20%	152
Sources: UNIP, Paris, France — EUROSTAT and national databases.						

Increased lupin area compensates for low yields

In 2005, average yields of sweet lupins in Europe were only 1.4 t/ha, 20% lower than the average for the last five years (Table 3). Thanks to the area increase of 22% compared with 2004, production remained stable at around 0.15 million tonnes. In Germany the main European producer, the lupin area increased by about 7%. Yields ranged from 1.32 t/ha in Brandenburg, the main producer with 55% of the German area, and 1.61 t/ha in Mecklenburg-Vorpommern to 3 t/ha in Nordrhein a small area in the West. In France lupin areas decreased by about 11% compared with 2004, but in Poland the lupin area increased by 150%.

Low production in 2005

The EU area dedicated to protein crops has remained stable at above 1.35 million hectares for the five last years, but actually, this relative stability has to be considered with caution. In East Europe after a marked decrease in the pea area there is now a tendency to stabilise or even to increase the area, but in North-West Europe, producing about 80% of the EU pea production, the tendency to reduce the pea area seems to be continuing with a loss of 26% of the area since 2000. The decrease in pea area has been compensated for partially by the southern countries, particularly Spain, which have shown a spectacular increase (about 236%) in the pea area during the same period but with lower yield potential. The increase in the faba bean area (+64%) is linked mainly to the well developed but limited Egyptian food market and does not compensate totally for the reduction in pea production.

This year, due to the bad yields registered particularly in France and Spain and to the medium yields registered in the other main producing countries, the production of protein crops in Europe was low (–10% compared with the average production over the last five years). Imports will certainly increase, in particular from Canada where the pea area (now 1.41 million ha) is still increasing (+5% over the last five years) and where the yields were almost as good as last year (2.37 t/ha).



Field pea and vetches in Serbia and Montenegro

Pois protéagineux et vesces en Serbie et Montenegro

by Vojislav Mihailović*, Aleksandar Mikić*, Branko Ćupina**and Pero Erić**

Terbia and Montenegro has a long tradition of cultivating field pea, vetches and other forage and grain legumes (4). Despite their significance, these species are grown on a relatively small area and to an insufficient extent. The most important grain legume is soyabean, being present among the Serbs since the beginning of the nineteenth century and with a harvested area of about 120,000 ha. As in other Balkan countries, the most significant pulse crop is *Phaseolus* beans which are cultivated on about 25,000 ha. Faba beans, lentil and grass pea are grown sporadically and without statistical data, while groundnut and chickpea, despite their wide use as snacks, are as a rule imported. Lupins, pigeon pea and cowpea are not familiar at all, with adzuki and mung beans found occasionally as healthy food and hyacinth bean as a purely decorative plant.

A long tradition with diverse utilisation

It is certain that some annual and grain legumes were well-known to the Slavs in their original homeland in eastern Europe more than fifteen centuries ago: for instance, the word for pea is the same in all present Slavonic languages, such as goroh in Russian, hrach in Czech and grašak in Serbian. Moreover, peas and vetches have been distributed widely over the Balkan Peninsula from time immemorial, since it was there that they entered Europe with wheat and barley. Peas in Serbia and Montenegro are grown on 40,000 ha, with field pea on 22,000 ha, forage pea on 4,000 ha and garden pea on 14,000 ha. Vetches are cultivated on about 7,500 ha.

The most ancient way of using peas and vetches is in the form of forage. There are

both winter and spring types, which can be grown alone or mixed with small grains, and they are cut at the full flowering or first pods stage, leaving enough time for maize, forage sorghum or Sudan grass as the next crop. Forage peas and vetches are also grown as green manure, while winter types are excellent cover crops for organic farming and sustainable agriculture (1).

Field pea was unknown in Serbia and Montenegro until twenty years ago, when it proved to be an excellent substitute for soyabean in dry seasons and quickly became better known as protein pea. With all the present cultivars being spring ones, field pea is usually grown alone and in a similar way to forage pea, except that the application of herbicides is necessary. The most important pest is pea weevil (Bruchus pisorum L.), requiring insecticides and an appropriate crop rotation. The most frequent diseases are powdery mildew (Erysiphe pisi DC.) in rainy seasons and by the end of maturing but without significant damage, and rust (Uromyces pisi DC.) and anthracnose (Ascochyta pisi Lib.), usually in dry seasons. Field pea production is still full of uncertainties for many farmers in Serbia and Montenegro. Some of them use non-certified seed, inducing wide propagation of pea weevil and non-uniform stands. At the same time, other crops such as winter wheat are often preferred, leaving peas and vetches to be grown on poor soil and in weedy fields lacking the inputs they require for good yields. In addition, the majority of pea cultivars mature at the same time as barley or wheat, which is another difficulty for farmers who have tended to lose interest in peas after only one season. However, progress in pea breeding and agronomy, and the recognised advantages of pea which include its short growing period, low inputs and its use for animal feed without heat treatments, continue to increase the number of farmers who are willing to face the disadvantages associated with field pea cultivation.

Vetches in Serbia and Montenegro are grown exclusively for forage, although many farmers have obtained good results when using the grain to feed pigs and poultry. There is no official data yet.

New varieties are being registered

The procedure of registration of new varieties of field pea and vetches in Serbia is supervised by the Ministry of Agriculture, Forestry and Water Management, with the network of field trials carried out at four sites over two years. Although Serbia and Montenegro is not a member of UPOV, a description of each candidate variety according to DUS tests1 is a necessary part of their application. In addition to the average yield, the criteria taken into account include date of flowering, date of cutting or harvest maturity, plant height, number of pods, thousand-seed weight, protein content, tolerance of low temperatures in winter types and lodging and susceptibility to diseases. There is no data on trypsin inhibitors yet. In 2004, an official Serbian national list contained 16 field pea, seven faba bean, four common vetch (Vicia sativa), one Hungarian vetch (Vicia pannonica), three hairy vetch (Vicia villosa), three lentil, two chickpea, one grass pea, one white lupin, one cowpea and one adzuki bean registered varieties (2).

Breeding programmes and research in Novi Sad

Apart from nearly seven decades of developing new cultivars of wheat, barley, maize, sunflower, sugar beet, soyabean and vegetable crops, the Institute of Field and Vegetable Crops in Novi Sad with its Forage Crops Department remains the leading institution in Serbia and Montenegro for breeding programmes on field pea, vetches and other forage and grain legumes (3). Novi Sad varieties are estimated to take 95% of the field pea and vetches area in the country. All breeding programmes are based upon the Annual Forage and Grain

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Table 1. Registered varieties of field pea and vetches developed at the Institute of Field and Vegetable Crops in Novi Sad.

Species and type	Variety	Year of registration and country	Yield of green forage (t/ha)	Yield of dry matter (t/ha)	Yield of grain (t/ha)
Winter forage pea	NS-Dunav	1977, S/M*	40.0-45.0	8.0-10.0	2.0-2.5
Winter forage pea	NS-Pionir	1977, S/M	45.0-50.0	10.0-12.0	2.0-3.0
Spring dual-purpose pea	NS-Junior	1992, S/M	40.0-50.0	9.0-12.0	3.0-4.0
Spring dual-purpose pea	NS-Lim	1992, S/M	35.0-40.0	7.0-8.5	3.0-3.5
Spring field pea	Moravac	1994, S/M			3.0-4.0
Spring field pea (afila)	Jezero	1995, S/M; 2005, Ukraine			4.0-5.0
Spring field pea	Javor	2002, S/M; 2005, Ukraine			4.0-5.0
Winter common vetch	Novosadska 624	1967, S/M	30.0-40.0	7.0-8.5	1.0-1.8
Winter common vetch	NS Sirmium	1979, S/M	30.0-45.0	7.0-9.0	1.0-1.6
Winter common vetch	Kadmos NS	1999, Greece	35.0-45.0	7.0-8.5	1.0-1.6
Winter common vetch	Neoplanta	2005, S/M	35.0-45.0	8.0-8.5	1.2-1.8
Spring common vetch	Novosadska 5590	1967, S/M	30.0-35.0	7.0-7.5	1.0-1.8
Spring common vetch	Beograd	1970, S/M	30.0-35.0	7.0-8.0	1.0-2.0
Spring common vetch	Novi Beograd	1997, S/M	35.0-40.0	7.0-8.0	1.0-2.5
Winter Hungarian vetch	NS Panonika	1979, S/M	35.0-40.0	6.0-7.5	1.2-1.8
Winter hairy vetch	NS Violeta	1979, S/M	35.0-45.0	8.0-9.5	0.8-1.2
*S/M = Serbia and Monteneg	ro				

Legumes Genetic Collection, with about 1,200 accessions of *Pisum*, *Vicia*, *Lupinus*, *Lathyrus*, *Lens*, *Cicer*, *Ornithopus*, *Vigna*, *Cajanus* and *Lablab* species.

The first breeding programme led to the development of the first domestic varieties of forage peas, with NS-Pionir, NS-Sirmium and Novi Beograd the most widely distributed varieties in the country. The goal of the second programme was the development of the first domestic spring pea dual-purpose varieties such as NS-Junior, since these were favoured by farmers wishing to supply the needs of both ruminants and non-ruminants with only one variety. The third programme produced the first domestic pea varieties solely for grain. With many advantages, such as lodging resistance and

decreased harvest losses, compared with the varieties of earlier generations, Jezero and Javor have become widely grown, leaving NS-Junior as the main spring forage pea variety. Among other breeding directions are the development of the first domestic winter pea cultivars for dual-purpose or grain, with early flowering and full maturity before winter wheat, the hybridisation between *Pisum sativum* and *Pisum fulvum*, in order to develop lines tolerant of pea weevil, and the development of the first domestic cultivars of vetches for grain, faba bean, grass pea, lentil, lupins and, if possible, cowpea, pigeon pea and hyacinth bean as well.

In addition to the breeding programmes, the Institute of Field and Vegetable Crops has a long tradition of cooperation with the Faculty of Agriculture in Novi Sad, especially for agronomy of annual forage and grain legumes and their utilisation. Both institutions have also been making the first attempts to preserve the genetic resources of annual forage and grain legumes, either at the national level or in joint projects such as the one with the Research Institute of Plant Production in Piešťany, Slovakia, due to start in 2006.

By joining the recently launched Technology Transfer Platform, an extension of the Grain Legumes Integrated Project, the Institute of Field and Vegetable Crops demonstrates that one of its strategic goals is to promote annual forage and grain legumes as a high quality and low cost means of meeting the constant demand for plant proteins in animal husbandry (5).



Trials with common vetch at IFVC, Novi Sad.

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- (4) Mihailović, V. et al. (2004). Grassland Science in Europe **9**, 457–459.
- (5) Mikić, A. et al. (2003). Biotechnology in Animal Husbandry 19, 465–471.
- ¹DUS is the Distinctiveness, Uniformity and Stability test issued by UPOV (International Union for the Protection of New Varieties of Plants).



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

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COVER PHOTO:

Seeds of diverse Leguminoseae in rows left to right and top to bottom: Vicia narbonensis, Vicia faba Troy, V. faba Portugese landrace, Lens culinaris LYDA-155, Cicer arietinum Castor, Arachis hypogaea EAN-198, Lupinus mutabilis Cortoz, Vigna unguiculata, Glycine max Protage, Phaseolus vulgaris, Vigna mungo, Lathyrus sativus. (Photos and seeds C. Chinoy, M. Ambrose, A. Davis, C. Domoney, JIC, Norwich UK)

PHOTO DE COUVERTURE:

Graines variées de la famille botanique Leguminoseae : de gauche à droite et de haut en bas : Vicia narbonensis, Vicia faba Troy, V. faba Portugese landrace, Lens culinaris LYDA-155, Cicer arietinum Castor, Arachis hypogaea EAN-198, Lupinus mutabilis Cortoz, Vigna unguiculata, Glycine max Protage, Phaseolus vulgaris, Vigna mungo, Lathyrus sativus. (Photos and seeds C. Chinoy, M. Ambrose, A. Davis, C. Domoney, JIC, Norwich UK)



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Special report

Dossier

Economic and environmental value of grain legumes

Valeur économique et environnementale des légumineuses à graines





AEP

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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.



Processors and Growers Research Organisation

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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.



Technical Institute for Cereals and Forage

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Run and financed by French farmers, Arvalis-Institut du végétal carries out and disseminates applied research on the production, storage and utilisation of cereals, grain legumes, potatoes, and forage.



UFOP

Union zu Förderung von Oel- und Proteinpflanzen

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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.