GRAIN LEGUMES

AEP

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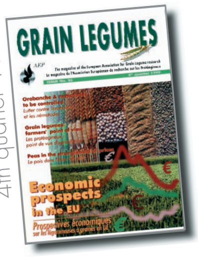

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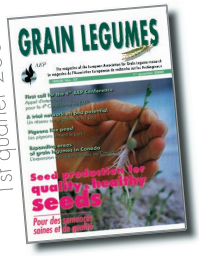

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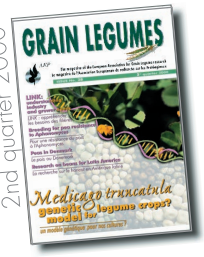

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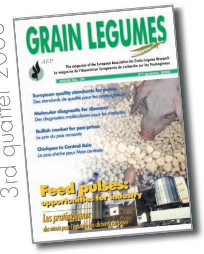

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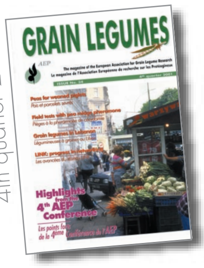

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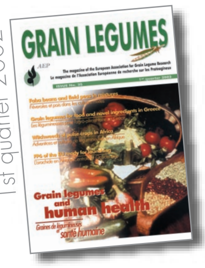

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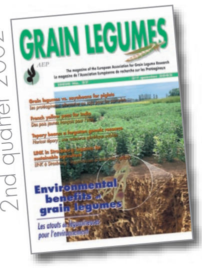

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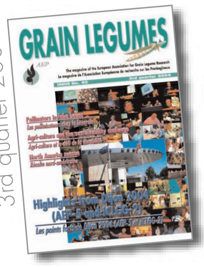

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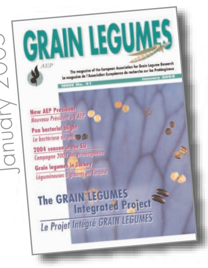

EDITORIAL

Disseminating information

I am honoured to be the AEP President for the period 2004–2007, and thus the current Publishing Director of Grain Legumes magazine. My first duty is to thank all the previous Presidents who have kept AEP very active in the legume world. Thanks to them, AEP has demonstrated that it has a key role in facilitating collaborations and information dissemination. The current EU Grain Legumes Integrated Project is very promising for the future of legume science.

Besides continuing to back R&D activities, AEP is currently concerned with communicating the results of research to those who will value them. Our magazine will help to disseminate scientific progress on legumes together with the web portal grainlegumes.com and the dissemination events.

We need to make industrialists, policy makers and citizens aware of the different benefits of introducing grain legumes in our agriculture, our animal feeding, our everyday meals, and in our industry, to contribute to healthy and sustainable systems.

Communicating is a challenge in our society, which is already over-saturated with information and where confusion occurs easily. Since legumes can really contribute to a more sustainable European society, we can be sure that we are on the right track.

Alvaro RAMOS MONREAL
AEP President and
Publishing Director of
Grain Legumes magazine

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Carte blanche to...

Geoffrey Gent*

Going for quality

The latest reform of the CAP has the potential to transform agriculture. With aid now being decoupled from production, farmers have to be satisfied that cropping plans will bring adequate reward for their investment, risk and commitment. Failing that, land will be withdrawn from agricultural use. In this context pulses have the potential to enhance farm profitability and make a positive contribution to the environment.

In the UK, profitability is often linked with production for value-added markets. These are diverse, but all depend on varieties that allow the production of unblemished grains of suitable size, shape, colour and cooking characteristics for particular markets. This emphasises the importance of plant breeding and the production of new varieties which combine excellence in both agronomic and quality characteristics.

The assessment of quality is complex, with seasonal growing and harvesting conditions often affecting the physical condition and culinary properties of both peas and beans. There is also the problem that requirements for some markets are subjective and variable according to supply and demand. However, through concentrating on human consumption quality, breeders can ignore some of the more difficult objectives for the animal feed markets such as high protein/high energy content.

Within the group of value added pulses, the large, dimpled, smooth-seeded marrowfat peas (1000-seed weight (tsw) 370 g) with good canning quality and colour are often the most rewarding for farmers. UK crops from the 2004 harvest can be three times the value of those for vegetable protein, amply compensating for their higher production costs and lower yields. Small-seeded conventional green peas (tsw 220 g) with good quality attributes for canning are in demand for human consumption in Southern Europe.

Quality attributes for beans are easier to define, for the value-added market is for human consumption in the Middle East. Here beans should be large-seeded (tsw 500g) pale-skinned with a white hilum and cook quickly for use over the Ramadan period. Other markets for very large-seeded beans (tsw 1000g) are developing. UK crops from the 2004 harvest with the necessary export quality have sold at a 20% premium over feed values, so emphasising the commercial value of these characteristics.

Quality crops should continue to reward growers, pulse processors and users and this will also mean that they are profitable for plant breeders. ■

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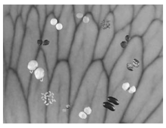

First landmark for the GRAIN LEGUMES Integrated Project

One year after its launch in Alicante (Spain), the Grain Legumes Integrated Project (1) held its annual meeting at the John Innes Centre, Norwich (UK) from 27 February to 1 March 2005. The purpose of the meeting was to share early results, forecast research activities in each project sub-area and increase interdisciplinary exchanges among the 150 scientists involved.

Scientific breakthroughs are expected to come from legume genetics and biology. A set of genomic and genetic resources are already developed for *Medicago truncatula* and for pea: the sequencing of the *M. truncatula* genome and the high-throughput analyses aiming to delineate gene function will allow a key set of genes to be identified. The first year of the project has brought progress in the development of plant mutant collections, oligo micro-arrays and bioinformatic data integration tools. These resources are currently being used to analyse the mechanisms involved in seed protein content, plant growth, and plant reaction to stresses such as diseases or pest attacks, soil salinity or drought conditions. Legume seed composition and properties will also be analysed extensively.

(Photo JIC, Norwich, UK)

Delegates at the Annual meeting of Grain Legumes Integrated Project.

Developments in comparative genetics among legume species (*M. truncatula,* pea, chickpea, faba bean, lentil, common bean, lupin, clover) are using information from models and applying it to cultivated species, in order to exploit genetic diversity and produce improved crop cultivars.

In parallel, the role of grain legumes in animal feed and also their uses in cropping systems are being assessed: analysis of their possible prebiotic role, analysis of the regional contexts for feed formulation and feed industry, measurements of environmental and economic benefits in the overall product life cycle, enhanced management for low input cropping systems. Intercropping trials have shown some positive impact for reduced disease pressure and enhanced plant nutrition in rotations.

It was also timely to present the Grain Legumes Technology Transfer Platform, which will assist the transfer of the combined results of scientific investigations related to these plants into commercial products. The GL-TTP is now open to membership from any interested parties such as scientific institutes, plant breeders and food and animal feed companies. ■

Source: A. Schneider, AEP, France. Email: a.schneider-aep@prolea.com

(1) For further details: GLIP is described in the special report of *Grain Legumes* **41**, 11–25; see also: www.eugrainlegumes.org

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Heritage and sustainable systems

Food safety and quality are topical issues for European agriculture and society. As an invited speaker at the GLIP Norwich meeting, Professor Jules Pretty, Director of the Centre for Environment and Society at the University of Essex (UK), pointed out how the current model, based on the intensive production of 'agri-commodities', is reaching its limits: the economic and environmental costs of transport in modern food systems are far-reaching and damaging, and the impact of diet on health in western countries is being questioned now that the costs of diet-related diseases exceed those of smoking.

Professor Pretty favoured a system of 'agri–culture', emphasising the importance of land and culture, and focusing on preserving and re-establishing the links between food consumers and their agricultural heritage. Every item of food has a story to tell and this story-telling is already developed to some extent for commodities such as cheese and wine. The 'agri-culture' concept is closely related to the implementation of a more sustainable agriculture. This is also an area where grain legume crops such as peas, faba beans and lupins, have a vital role to play in the sustainability of cropping systems and the production of food raw materials. ■

See also the article by Jules Pretty in Grain Legumes Issue No. 40, 22–23.

Australian Pulse Breeding Programme – future direction

In Australia a new National Pulse Breeding Programme will unite field pea, chickpea, faba bean and lentil breeding programmes, currently operating across the five states. This will provide an internationally competitive breeding capability for the different pulses that meets the regional needs of Australian pulse growers and the marketing sector.

A committee, comprising the Grains Research and Development Corporation (GRDC), Pulse Australia and major state-based pulse breeding agencies, that has been meeting to consider the options, wants a national programme for pulse breeding that can achieve efficiencies through greater integration and collaboration at the national level, whilst recognising the need for specific regional and end-user requirements. Such a national programme would also build on pre-breeding, breeding, regional evaluation and market information to maximise the benefits from investment in pulse research by the various partners.

Announcing the national programme, GRDC Managing Director, Peter Reading, said a sustainable future for Australia's 40,000 grain growers and their current A$8 billion per annum industry was closely tied to pulse crops.

"For this reason, it is vital that breeding programmes share germplasm, technologies and intellectual property, so that benefits flow freely across state borders.

With around A$10 million invested annually in the different pulse breeding programs in WA, SA, Victoria, NSW and Queensland, it makes sense to encourage greater sharing of individual resources for the common good, while ensuring a clear focus remains on local objectives being satisfied," he said.

The National Pulse Breeding Programme is expected to be functioning by July, 2005. Further details can be found on the GRDC web site at http://www.grdc.com.au/news/pulse.htm ■

Source: GRDC media release 17 February 2005.

FP7 preparation

On 6 April 2005 the European Commission announced its proposals for the Seventh Framework Programme for research and development (FP7, 2007-2013) (COM(2005) 119 final). FP7 is to be launched at the end of 2006.

Four major objectives

The FP7 (total budget €72,726 million) will be organised in four specific programmes, corresponding to four major objectives of European research policy:

Cooperation: Support will be given to the whole range of research activities carried out in trans-national cooperation, from collaborative projects and networks to the coordination of research programmes. International cooperation between the EU and third countries is an integral part of this action. (€44,432 million)

Ideas: An autonomous European Research Council will be created to support investigator-driven 'frontier research' carried out by individual teams competing at the European level, in all scientific and technological fields, including engineering, socioeconomic sciences and the humanities. (€11,862 million)

People: The activities supporting training and career development of researchers, referred to as 'Marie Curie' actions, will be reinforced with a better focus on the key aspects of skills and career development and strengthened links with national systems. (€7,129 million)

Capacities: Key aspects of European research and innovation capacities will be supported: research infrastructures; research for the benefit of SMEs; regional research-driven clusters; unlocking the full research potential in the EU's 'convergence' regions; 'Science in Society' issues; 'horizontal' activities of international co-operation. (€7486 million)

Food, Agriculture and Biotechnology – one of nine themes

Among the nine operationally autonomous but cooperating sub-programmes, 'Food, agriculture and biotechnology' is defined as 'Building a European Knowledge Based Bio-Economy by bringing together science, industry and other stakeholders, to exploit new and emerging research opportunities that address social and economic challenges'.

Its activities are:

Sustainable production and management of biological resources from land, forest, and aquatic environments: Enabling research, including 'omics' technologies, such as genomics, proteomics, metabolomics, systems biology and converging technologies for micro-organisms, plants and animals, including exploitation of their biodiversity; improved crops and production systems, including organic farming, quality production schemes and GMO impacts; sustainable, competitive and multifunctional agriculture, and forestry; rural development; animal welfare, breeding and production; plant health; sustainable and competitive fisheries and aquaculture; infectious diseases in animals, including zoonoses; safe disposal of animal waste; conservation, management and exploitation of living aquatic resources, developing the tools needed by policy makers and other actors in agriculture and rural development (landscape, land management practices etc.).

'Fork to farm': food, health and well being: Consumer, societal, industrial and health aspects of food and feed, including behavioural and cognitive sciences; nutrition, diet related diseases and disorders, including obesity; innovative food and feed processing technologies (including packaging); improved quality and safety, both chemical and microbiological, of food, beverage and feed; integrity (and control) of the food chain; environmental impacts on and of food/feed chains; total food chain concept (including seafood); traceability.

Life sciences and biotechnology for sustainable non-food products and processes: Improved crops, feed-stocks, marine products and biomass (including marine resources) for energy, environment, and high added-value products such as materials and chemicals, including novel farming systems, bio-processes and bio-refinery concepts; bio-catalysis; forestry and forest based products and processes; environmental remediation and cleaner processing. ■

Source: EUROPA.
For more details:
http://www.grainlegumes.com/default.asp?id_biblio=360&rub=link
or http://www.cordis.lu/fp7/

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"I underline that the proposals are doubling the EU budget for research and development on an annual basis [. . .]. What we need now is to get on with finalising the framework programme, and more urgently, making sure that sufficient funds will be available."

Janez Potocnik, the Science and Research Commissioner

Western Australian lupin pathologist awarded National Medal

Dr Mark Sweetingham a plant pathologist with the Western Australian Department of Agriculture who works predominantly on pathogens of narrow-leaved lupin (*Lupinus angustifolius*), has just been awarded the Australian Institute of Agricultural Science and Technology Medal of Agricultural Science for 2005.

Mark has published extensively on diseases of lupins. He worked on lupin anthracnose (*Colletotrichum lupini*) following its discovery in Western Australia. The disease Phomopsis stem blight (PSB) caused by *Diaporthe toxica,* which causes minor damage to the crop but can cause numerous deaths of sheep grazing lupins, has been another of his research areas. In addition he has worked on both *Rhizoctonia solani* and *Pleiochaeta setosa,* the causes of brown spot and root rot of narrow-leaved lupin.

Mark works closely with plant breeders on the use of biotechnology to confer disease resistance. He has also been instrumental in the development of screening techniques for a variety of lupin diseases. ■

Source: G. D. Hill, Lincoln University, New Zealand.
Email: hill1@lincoln.ac.nz

International Lupin Association holds 11th meeting in Mexico

The International Lupin Association has just held its 11th meeting in Guadalajara, Mexico on 4–9 May 2005. The meeting was hosted on behalf of the Association by the Centro Universitario de Ciencias Biológicas y Agropecuarias of the Universidad de Guadalajara.

Papers presented at the lupin conference vary with the different nations hosting the conference. At this meeting a number of papers were presented on a range of wild lupin species from Mexico, which are currently under investigation for their potential.

The advantage of the addition of lupin kernel fibre in the human diet, indicated at the 10th meeting in Iceland was reinforced by a paper from Australia. Other papers in a session on 'Lupins and health' also dealt with the advantages of eating lupin seed.

The disease anthracnose (*Colletotrichum lupini*) is still a major problem engaging the attention of plant pathologists and breeders. In some parts of the world lupins are now more interesting because of their potential for sustainable and organic farming systems. Lupin breeding currently appears to be concentrating on improved disease resistance. However, workers in Portugal are looking at selections in Lupinus cosentinii, which is naturalised in south-western Australia, for more extensive use under Mediterranean conditions.

The conference was not all work and before it ended delegates were taken to the town of Tequila where they were instructed on the finer points of Tequila making and drinking.

There is a large poultry industry located near the city of Guadalajara, which currently relies on imported soyabean meal as a major protein supplement. Growing lupin seed has the potential to provide a locally produced protein source for poultry rations.

At the International Lupin Association General Assembly, held during the Conference it wad decided that in 2008 the 12th meeting of the Association would be held in Western Australia, the world's largest producer of lupins. By that time it will be 22 years since the Association met in 1986 in Geraldton. ■

(Photo: G. D. Hill, Lincoln University, New Zealand)

Delegates at the 11th International Lupin Conference.

Source: G. D. Hill, Lincoln University, New Zealand.
Email: hill1@lincoln.ac.nz

PhD theses

Modification of the proteome and variations in the composition of metabolites: soluble sugars, starch, organic acids and proline during acclimatisation to cold associated with frost tolerance in peas*

Modification du protéome et variations de la composition en metabolites: sucres soluble, amidon, acides organique et proline, au cours de l'acclimatation au froid associées à la tolerance au gel du pois

by Sophie ALVAREZ**

A possible relationship between the acquisition of frost tolerance and the levels of soluble sugars, starch, organic acids and proline were examined in two pea (*Pisum sativum* L.) cultivars: cv. Champagne (frost tolerant) and cv. Térèse (frost sensitive). The content of sucrose and raffinose in the shoots and roots of Champagne plants increased to significantly higher levels in response to cold acclimatisation. However, in 59 recombinant inbred lines derived from a cross between Champagne and Térèse, and grown in the same field trial, accumulation of soluble sugars during cold acclimatisation did not guarantee frost tolerance. In parallel, proteome analysis of proteins from buds, leaves, stems and roots of Champagne and Térèse during cold acclimatisation showed the specific expression of protein associated with the photosynthetic apparatus and involved in the repression of oxidative stress in Champagne. ■

*PhD thesis 2004, Université des Sciences et technologies de Lille.
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Digestion of pea proteins in weaned piglets and growing pigs. Characterisation of peptides resistant to digestion*

Digestion des protéines de pois chez le porcelet et le porc en croissance. Caractérisation des peptides résistants à la digestion

by Maud LE GALL**

The incorporation of pea in animal feed is limited by the low nutritional quality of the raw meal. This has long been ascribed to the presence of a limiting amount of essential sulphur amino acids and the poor digestibility of proteins caused by the presence of antinutritional factors in the seed.

Our studies showed that the major pea storage proteins, globulins, are well digested in the pig. The α polypeptide of legumin is totally hydrolysed by pepsin, while the β polypeptide and vicilin are digested by pancreatic and intestinal enzymes only. Proteins from the albumin fraction are more resistant to digestion. The lectin and the albumin PA1b are totally resistant in the gastrointestinal tract. By contrast the susceptibility of the major albumin PA2 to digestion is influenced by different factors. It is totally hydrolysed by pepsin while being partly resistant to pancreatic and intestinal enzymes. Consequently, for a gastric retention time below three hours, a cleaved PA2 peptide of 15 kDa escapes gastric and small intestinal digestion. Pea particle size reduction and heating enhance susceptibility to digestion by increasing protein accessibility to enzymes. ■

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Grain legumes to fight hypercholesterolaemia

Les légumineuses pour combattre l'hypercholestérolémie

by Anna ARNOLDI*

It has become urgent to promote a more appropriate diet in industrialised countries, because the ageing population has increased the incidence of cardiovascular disease, obesity, and type II diabetes. The beneficial role of a regular consumption of grain legumes in the prevention of these pathologies is considered based on the recent literature.

Soyabean proteins prevent cardiovascular disease

Many experimental studies have demonstrated that soya proteins reduce plasma cholesterol both in animals and in hypercholesterolaemic patients. The first clinical study was performed in Italy (9) and showed a 20–22% reduction of total cholesterol and a 22–25% fall in low-density-lipoprotein (LDL) cholesterol in hypercholesterolaemic subjects who had consumed soyabean proteins instead of animal proteins (i.e. meat, eggs, and dairy products) for some weeks. Studies have shown that hyperlipidaemic patients give the most favourable response when animal proteins are replaced by soyabean proteins. At present, the soyabean diet is certainly the most effective dietary tool for treating hypercholesterolaemia (1) and provides a unique opportunity for the management of very young patients (10). Consequently, in 1999 the US Food and Drug Administration validated the health claim for the role of soy protein in reducing the risk of coronary heart disease, mainly by reducing cholesterolaemia.

Grain legumes may prevent cardiovascular disease

Since the 7S globulins are the active components of soyabean (10) and in view of the high homology of grain legume proteins, it is reasonable to foresee that other grain legumes may exert a protective activity similar to soyabean. Available studies in this field are still scarce, but quite promising.

Table 1, adapted from reference (3), summarises the most significant experimental studies published to date on whole meals or protein isolates from grain legumes. They were produced using the classical animal model for hypercholesterolaemia, i.e. the rat fed a diet very rich in cholesterol, cholic acid, and coconut fat, using milk proteins (lactalbumin or casein) as the reference animal proteins. All the tested protein isolates or meals were effective in reducing total and LDL-cholesterol. Chickpea and common bean meals appear to be the most effective, but the comparison is not easy, because the data, produced in slightly different experimental conditions, are not homogeneous. However, Table 1 suggests clearly that most grain legumes may be beneficial in the prevention of cardiovascular disease.

Functional food ingredients

The functional food market requires very effective and economic food ingredients. Therefore, only legumes with a very high protein content are likely to be realistic competitors of soyabean. While most legumes contain only 20–25% protein and about 45–55% starch, the only protein-rich legume besides soyabean is lupin, whose protein content may reach 35–40% depending on the species. Only lupin seeds have the characteristics to become a feasible source of protein food ingredients and so its hypocholesterolaemic activity requires a detailed description.

The effect of blue lupin *(Lupinus angustifolius)* was compared with that of lactalbumin (8). A significant lowering effect on total plasma cholesterol was observed in rats fed the seed meal or five different semi-purified lupin fractions. The most effective fraction, containing γ-conglutin, lowered total plasma cholesterol by 34% compared with lactalbumin. In addition, liver lipid and cholesterol were also decreased.

Table 1. Relevant studies on rats fed cholesterol-rich diets based on protein isolates or meal from grain legumes (adapted from (3)).

Legume	Preparation	% change of total cholesterol	% change of LDL-cholesterol	Literature
Soyabean	Protein isolate	–22.70	n.d.	(5)
White lupin	Protein isolate	–22.69	–30.16	(11)
Blue lupin	Protein isolate	–29.08	n.d.	(8)
Common bean	Protein isolate	–26.88	n.d.	(6)
Faba bean	Protein isolate	–29.89	–36.61	(7)
Common bean	Meal	–36.31	–52.99	(4)
Pea	Meal	–13.99	–26.90	(4)
Lentil[1]	Meal	– 6.70	–32.24	(4)
Lima bean[2]	Meal	–23.96	–38.81	(4)
Chickpea[3]	Meal	–34.06	–43.17	(12)

n.d. = not determined.
[1] *Lens culinaris;* [2] *Phaseolus lunatus;* [3] *Cicer arietinum.*

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Table 2. Clinical studies on pulses: serum lipids responses of hypercholesterolaemic or mild hypercholesterolaemic subjects (adapted from (3)).

			Total cholesterol		LDL – cholesterol	
Entry	Type of pulse	Amount (g/day)	Control value (mg/100 mL)	Change %	Control value (mg/100 mL)	Change %
1	Common beans	120	298	-18.5	220	-23.12
2	Common beans	69	295	-10.4	200	-8.4
3	Mixed beans	140	268	-7.1	188	-4.80
4	Beans	145	244	-0.47	179	-0.43
5	Beans + oat bran	80	242	-0.80	162	0.00
6	Faba beans	90	240	-6.90	169	-7.32
7	Common beans	110	237	0.00	188	+4.52

White lupin *(Lupinus albus)* has attracted our attention recently (11), because this legume, used in the Mediterranean area from very ancient times, has all the characteristics to make it acceptable to European consumers. Our study applied a pharmacological approach: rats were fed a hypercholesterolaemic diet containing 1% cholesterol, 0.5% cholic acid, and 20% casein, and force fed with lupin protein isolate at 50 mg/rat for 14 days. Lupin-treated rats showed a 23% total cholesterol and a 30% LDL-cholesterol reduction. It must be stressed that this daily dose was particularly low, comparable with some well-known lipid lowering drugs, such as fibrates. We also demonstrated that some lupin protein fractions are able to up-regulate the LDL receptors in liver cells. This has been demonstrated to be the main mechanism of action of soyabean proteins (10). Certainly white lupin is a promising source of ingredients for the preparation of hypercholesterolaemic functional foods.

Clinical studies are promising

Available clinical data have been reviewed recently (2, 3): there are eleven studies, but five have involved only normolipidaemic subjects and will not be taken into consideration here. So far, only common bean *(Phaseolus vulgaris)* and faba bean *(Vicia faba)* have been investigated. Other legumes, including lupin, are still to be examined in detail, and work in this area is in progress in our laboratory.

The most relevant results of these clinical studies are summarised in Table 2. Positive results were observed only in four studies, 1, 2, 3, and 6: as in the case of soyabean protein, a clear reduction in total and LDL-cholesterol is observed only when the dietary intervention involved hyperlipidaemic subjects, whereas studies on borderline subjects were ineffective.

Study 1 was based on *Phaseolus* beans and involved very hyperlipidaemic subjects: after 21 days the bean diet (daily intake of dry pinto or navy beans = 115 g, served as cooked beans or soup) decreased total cholesterol concentration by 18.5% and LDL-cholesterol by 23.1%.

Study 2 was carried out on hyperlipidaemic subjects, who consumed canned *Phaseolus* beans for 21 days. Diet 1 included 120 g beans daily, whereas diet 2 included 162 g beans: both diets were effective, lowering total and LDL-cholesterol and triglycerides by about 10%.

In study 3, seven male mildly hyperlipidaemic patients consumed approximately 140 g tinned mixed beans daily over a 4-month period. After this, triglycerides were reduced by 25%, while total and LDL-cholesterol levels were reduced by 7%.

Study 6 examined the effects of faba bean *(V. faba)* flour in young men (aged 18–21 years) with borderline hypercholesterolaemia (average cholesterol 240 mg/dl). All subjects consumed the same basic diet and in addition the control group A consumed 90 g wheat flour daily, whereas group B consumed 90 g cooked faba bean flour. After 30 days, total cholesterol, LDL-C, and VLDL-C, triglycerides, glucose and insulin values were lower than the initial ones in all subjects of group B.

The experimental designs of these studies were very different, but they were all based on whole seeds, thus not helping in singling out which component(s) is (are) responsible for the observed effects. The following order of importance has been proposed (2): soluble dietary fibre, proteins, oligosaccharides, isoflavones, phospholipids, fatty acids, phytosterols, saponins or, possibly, other not yet recognised factors. The structural similarity of grain legume 7S globulins and the few experimental studies on protein isolates (Table 1) suggest that grain legume proteins, like soyabean proteins, may have a very relevant role. Therefore it can be concluded that pulses may be very beneficial in the prevention of cardiovascular disease and physicians and nutritionists should encourage their consumption as substitutes for animal proteins. ■

Acknowledgement. Work supported by the European Commission: project Healthy-Profood (QLRT 2001-2235).

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Genetic diversity in Spanish grain legume collections

Diversité génétique des collections espagnoles des légumineuses à graines

by Lucía DE LA ROSA*, Isaura MARTÍN, Federico VARELA and Celia DE LA CUADRA

The Mediterranean Basin is a centre of origin for many different grain legume species and the Iberian Peninsula is reported to be an area of diversification for some *Vicia* species such as *V. faba, V. articulata* (6) and *Phaseolus vulgaris* (5). Grain legumes, together with cereals, have been the basis of Spanish traditional agriculture for centuries. This could be the reason for the enormous genetic diversity present in Spanish grain legumes. Since the middle of the twentieth century, however, the cultivation of some grain legume species, especially minor species such as *V. articulata, Lathyrus sativus* or *Lathyrus cicera,* has suffered a serious decline in Spanish agriculture. For a few species, such as *V. sativa,* the downward trend in the growing area was reversed following changes in the Common Agricultural Policy in 1994. In these cases, the use of commercial varieties obtained from other countries, and not adapted to Spanish agro-climatic conditions, has had a negative effect on the richness of agrodiversity.

Avoiding an irreversible loss of diversity

Severe genetic erosion of Spanish grain legumes has been evoked but, fortunately, the irreversible loss of diversity has been partly avoided by the collection and *ex situ* conservation of genetic resources in germplasm banks. These activities have continued to allow their utilisation by farmers, breeders and researchers.

The Spanish Plant Genetic Resources Centre, belonging to the National Institute for Agricultural Research (CRF-INIA) is responsible for the following topics included in the National Programme for Conservation and Utilisation of Plant Genetic Resources for Food and Agriculture (PGRFA):

- To act as the Documentation Centre for the Plant Genetic Resources network, and to implement the National Inventory;
- To hold the National Seed Base Bank and be responsible for maintaining a safe duplicate of the samples under long-term conservation;
- To keep active (exchangeable material) collections of any species not included in another active bank.

At present, in Spring 2005, the Spanish Plant Genetic Resources Inventory includes 61,900 accessions. The database is organised according to the FAO/IPGRI *Multi-crops Passport Descriptors* (www.ecpgr.cgiar.org) and it is available online (www.inia.es). This inventory records 14,110 grain legume accessions, 8470 of them collected in Spain. Twelve institutions hold the collections (Table 1).

Grain legume collections at CRF

Grain legumes are the largest active collection maintained at CRF and management of this collection is one of its most important responsibilities. At present, the collection comprises 10,133 accessions, 6717 of Spanish origin and mostly landraces. Table 2 shows this collection grouped by species and type of germplasm, separating landraces and wild material. Figure 1 shows the distribution of accessions by Autonomous Regions. Some of these accessions came from expeditions organised by CRF. Since 1986 CRF has conducted collecting missions in 25 Spanish provinces. The total number of accessions collected was 5225, 1804 of them grain legumes. This material, together with accessions from previous expeditions and/or donations, has resulted in a good representation of the genetic variability of Spanish grain legumes. CRF manages most of the genus as an active collection and maintains a duplicate as a base collection. Some active collections are in the process of being transferred to others institutions for different reasons. *Lupinus*, with soil requirements different from those of Central Spain, has been transferred to the Food and Agricultural Service of Research, Development and Technology (SIDTA, Badajoz) and the *Lens* collection is in the process of transfer to the Service for Agricultural Research (SIA, Cuenca) where a lentil breeding team is working with this species. Spanish beans exhibit great diversity, and accessions of this crop used for the local market or for home consumption have been obtained during the majority of collecting trips. Bean multiplication/regeneration and characterisation are very expensive and time consuming and, for this reason, a network formed by Spanish research institutions and universities has been established.

Table 1. Spanish institutions holding grain legume collections.

FAO code	Institution	Species
ESP003	UPM, Madrid	Multi-crops
ESP004	CRF-INIA Alcalá de Henares, Madrid	Multi-crops
ESP009	MBG-CSIC, Pontevedra	*Phaseolus, Pisum, Vigna*
ESP010	SIDTA-La Orden, Badajoz	*Lupinus*
ESP016	NEIKER- Arkaute, Alava	*Phaseolus*
ESP026	UPV-COMAV, Valencia	Multi-crops
ESP027	SIA-BGH-Zaragoza	Multi-crops
ESP032	SERIDA-Villaviciosa, Asturias	*Phaseolus*
ESP036	ITA- Zamadueñas, Castilla y León	*Phaseolus, Pisum, Vicia*
ESP046	IFAPA-Córdoba	*Cicer, Vicia*
ESP119	SIA-Mabegondo, La Coruña	*Phaseolus, Pisum*
ESP124	SIDTA-Albadalejito, Cuenca	*Lens, Vicia*

Storage conditions

Most of the legumes have orthodox seed storage behaviour. This means that their longevity

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Figure 1. The CRF grain legumes collection, number of accessions distributed by region of origin.

can be prolonged considerably under conditions of low temperature and internal moisture. Seeds are desiccated to an internal moisture below 7% and stored both in hermetically closed tins at –18 ºC for long-term conservation (Base Bank) and in openable glass jars at –4 ºC for medium conservation term (Active Bank).

The initial seed viability is determined by a germination test and then monitored every ten years thereafter. The germination test should be adapted as necessary for different species. Large-seeded species such as *Phaseolus vulgaris, Vicia faba* or *Lathyrus sativus,* should be hydrated slowly to avoid internal damage. Hard seeds of some species (genus *Vicia* or *Lathyrus)* are scarified manually making a small incision in the seed coat. For small-seeded species different scarification treatments are being tested (4). Germination of at least 85% makes the accession acceptable for Bank introduction.

The genetic diversity of grain legumes has been described mainly by agro/morphological traits, based on the IPGRI (International Plant Genetic Resources Institute) descriptor lists. In the case of *V. articulata V. narbonensis* and *Lathyrus sativus* characterisation descriptor lists had to be developed. The number of accessions by species and traits studied (in brackets) are: *Cicer arietinum* 613 (13), *Lathyrus cicera* 162 (23), *Lathyrus sativus* 61 (18), *Lens culinaris* 335 (17), *Lupinus albus* 500 (17), *Phaseolus vulgaris* 2498 (14 seed traits) and 619 (12 agro-morphological traits), *Pisum sativum* 290 (22 agro-morphological traits and several ISSR-PCR – Inter Simple Sequence Repeats PCR-molecular markers), *Vicia articulata* 78 (12), *Vicia ervilia* with two groups of data, one of 203 (18) characterised at CRF and another group with 219 (7) characterised at the regional institution of Castilla La Mancha (ITAP), *Vicia faba* 550 (12), *Vicia narbonensis* 15 (13), *Vicia sativa* 500 (30) and *Vicia villosa* 29 (10).

In the case of peas (1) and beans (3) sub-collections representative of the diversity of the whole collection (core collections) have been established. Currently, a characterisation of the *Phaseolus* core collection is in progress using morphological traits and seed reserve proteins. Some of this information is on the INIA web site (www.inia.es).

Habitually, the users of the grain legumes collection were mostly breeders or researchers, but in recent years the utilisation of landraces on farms again has become an important aspect of PGR's activities (2). In a project carried out by CRF, a multi-disciplinary team was established together with farmer associations, including organic farmers, local organisations and agronomy researchers in order to promote the collection of some landraces of *Lens culinaris* in an agricultural area in central Spain.

There is no doubt that the Spanish legumes collection will continue to be a valuable source of genetic diversity for research and breeding for years to come. ■

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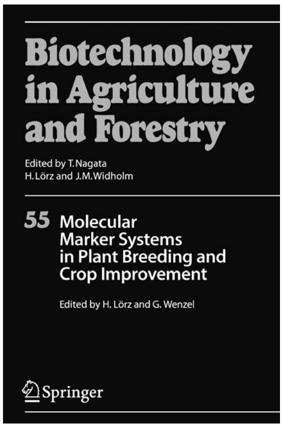
Table 2. The grain legumes collection at CRF.

Species	Landraces	Wild
Cicer arietinum	338	
Dolichos lablab	4	
Lathyrus cicera	160	13
Lathyrus sativus	157	
Other *Lathyrus*	3	36
Lens culinaris	286	
Other *Lens*		19
Lupinus albus	228	6
Lupinus angustifolius	1	486
Lupinus hispanicus		90
Lupinus luteus	7	98
Other *Lupinus*		98
Phaseolus vulgaris	2462	
Other *Phaseolus*	80	
Pisum sativum	420	2
Trigonella foenum-graecum	10	
Vicia articulata	95	2
Vicia ervilia	163	
Vicia faba	724	
Vicia narbonensis	9	11
Vicia sativa	355	131
Vicia villosa	16	28
Other *Vicia*		57
Vigna unguiculata	70	

(Photo L. de la Rosa, CRF-INIA, Spain)

Left to right and top to bottom:* CRF grain legumes accessions: *Lathyrus clymenum, Vicia lutea, Lupinus mariae-josephi* and *Vicia atropurpurea.



Molecular marker systems in plant breeding and crop improvement

Series: Biotechnology in agriculture and forestry, Vol. 55

H. Lörz and G. Wenzel (Eds)

2005, English, 476 pages, 42 illustrations, 5 colour

ISBN: 3-540-20689-2

Successful release of new and better crop varieties increasingly requires genomics and molecular biology. This volume presents basic information on plant molecular marker techniques from marker location up to gene cloning. The text includes a description of technical approaches in genome analysis such as comparison of marker systems, positional cloning, and array techniques in crop plants including alfalfa, pea and *Vigna*. A special section focuses on converting this knowledge into general and specific breeding strategies, particularly in relation to biotic stress. Theory and practice of marker assisted selection for QTL, gene pyramiding and the future of MAS are summarised and discussed for maize, wheat and soyabean. The volume ends with a comprehensive review of the patents relevant for using molecular markers and marker assisted selection.

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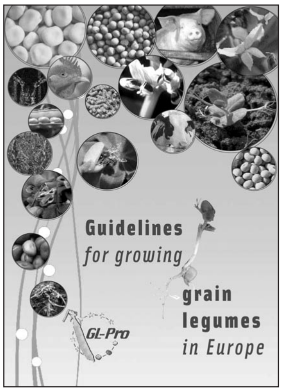

Growing grain legumes in Europe

May 2005, English, French, Spanish, German, 8 pages

There is great potential for the expansion of grain legumes in Europe, which is why a European network of crop experts has been brought together in the GL-Pro project[1] and is being reinforced progressively.

'Guidelines for growing grain legumes in Europe', a colourfully-illustrated leaflet about growing grain legumes has been produced in four languages as a result of collaborations between the project partners.

The leaflet is aimed at technical advisers, farmers and decision-makers providing them with an easy to read synopsis of the key facts about growing grain legumes in Europe. The nine major types of grain legume crops are illustrated and their potential growing areas are shown on a series of maps. Suitable soils and climatic constraints are summarised for all nine crops and the agronomic, environmental and economic benefits are outlined. A table shows the composition of the different legume seeds and the value of a given legume species as raw material for feed rations for a given animal is assessed.

Contact persons and organisations providing further technical information and advice on cropping management at the local level are listed for eight countries.

To get free copies of the leaflet: g.dubois@prolea.com

[1] GL-Pro (2003–2006) European extension network for the development of grain legume production in the EU. http://www.grainlegumes.com/gl-pro/

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SEPTEMBER 22–23, 2005
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Drought and saline stress in legumes

Les légumineuses face à la sécheresse et la salinité

(Photo J.-J. Drevon, INRA Montpellier, France)

Morphological aspects of *Phaseolus vulgaris* plants, line BAT 477, after five weeks of saline treatment.

Abiotic stresses such as drought or salinity are interconnected with secondary stresses such as osmotic and oxidative stress. In tolerant plants, after the initial stress signals the downstream process involves transcription controls activating stress-responsive mechanisms that maintain or re-establish ion homeostasis, facilitate retention and/or acquisition of water, protect chloroplast functions and membrane integrity (1). In legumes, because soil related stress interacts with root systems and nodules, the specific relations with symbiotic nitrogen fixation require a special focus.

The first article of this special report summarises the efforts to target common beans better adapted to moderate salinity in the Mediterranean region. Then the case of chickpea illustrates the search for combinations of 'plant genotype x bacteria strain' to achieve higher rates of symbiotic nitrogen fixation under such conditions. The stress adaptation has highlighted the key role of compatible solutes in osmoregulation in plants but also in the bacteria, and this is the subject of the third article.

A new international project, which aims to increase the production of grain legumes in Mediterranean countries where yields are affected by water deficit, will contribute to research progress on symbiotic associations in these conditions. The knowledge on pea crop physiology under water deficit in European conditions is also summarised. The high-throughput analysis of genes and molecules activated in a stress-adaptive response is useful to decipher further the pathways involved: on-going research using the model legume *Medicago truncatula* is described.

(1) Vinocur, B. and Altman, A. (2005). Recent advance in engineering plant tolerance to abiotic stress: achievements and limitations. Current Opinion in Biotechnology **16**, 123–132.

Les pressions environnementales telles que la sécheresse et la salinité sont en étroite interaction avec les stresses secondaires comme le stress osmotique et oxydatif. Dans le cas des plantes tolérantes, les processus déclenchés par les premiers signaux impliquent des contrôles de la transcription activant les mécanismes de réponse au stress qui maintiennent ou rétablissent l'homéostasie, facilitent la rétention ou l'accumulation d'eau, protègent le fonctionnement du chloroplaste et l'intégrité de la membrane cellulaire (1). Chez les légumineuses, comme les stresses abiotiques interagissent avec le système racinaire et les nodules, les relations avec la fixation azotée symbiotique doivent avoir une attention particulière.

Le premier article de notre dossier résume les travaux portant sur une meilleure adaptation du haricot aux conditions modérées de salinité en région méditerranéenne. Le cas du pois chiche illustre ensuite la recherche du couple 'génotype végétal x souche bactérienne' permettant une meilleure fixation azotée dans ces conditions. L'adaptation au stress a montré l'importance du rôle des solutés compatibles dans la régulation osmotique au sein des cellules végétales mais aussi chez les bactéries ce qui est le sujet du troisième article.

Un nouveau projet, qui vise à augmenter la production des légumineuses à graines dans les pays Méditerranéens où les rendements sont affectés par le déficit en eau, va apporter de nouveaux éléments de réponse sur la meilleure association symbiotique en conditions difficiles. Les connaissances accumulées sur l'agro-physiologie du pois face au stress hydrique en conditions européennes sont également résumées. L'analyse à grande échelle de l'expression des gènes et molécules actifs en cas de réponse adaptative au stress est utile pour disséquer plus précisément les métabolismes impliqués: les travaux en cours sur la plante modèle Medicago truncatula *sont présentés.*

*(1) Vinocur, B. and Altman, A. (2005). Recent advance in engineering plant tolerance to abiotic stress: achievements and limitations. Current Opinion in Biotechnology **16**, 123–132.*

Salinity adaptation of *Phaseolus* beans in FABAMED-related activities

Adaptation du haricot aux conditions salines modérées dans le cadre des activités FABAMED

by Jean-Jacques DREVON*

Legume–rhizobia symbiosis should be a priority area for research because of the important part it plays in fixing atmospheric nitrogen (N) for the production of protein-rich seeds and fodder and its beneficial effect on crop productivity in diverse cropping systems around the planet. Thus, in 1995, FABAMED was created as a cooperative research group with the general objective of applying some of the spectacular advances in methodologies and knowledge of symbiotic nitrogen fixation (SNF) to improve the N_2-dependent growth of legumes and stabilise their productivity under the pedoclimatic limitations of the Mediterranean Basin. FABAMED agronomists, plant breeders, microbiologists, physiologists and molecular scientists meet annually to revise the interdisciplinary strategy through on-going projects and new proposals for bi- and multi-lateral cooperation. It addresses a range of tasks arranged under four disciplines: agronomy, biodiversity, physiology, and genetics–genomics (4, 6, 12).

This interdisciplinary strategy (Figure 1) is illustrated here with results obtained by FABAMED for adaptation to moderate salinity of nodulated common bean (*Phaseolus vulgaris*) as a model legume crop for improvement of legume cultivation in salinised Mediterranean areas. Indeed, more than 40% of soils in the Mediterranean Basin are saline and this limits legume productivity considerably, whereas draining salinised soils with good quality water, generally from remote sources, is very costly.

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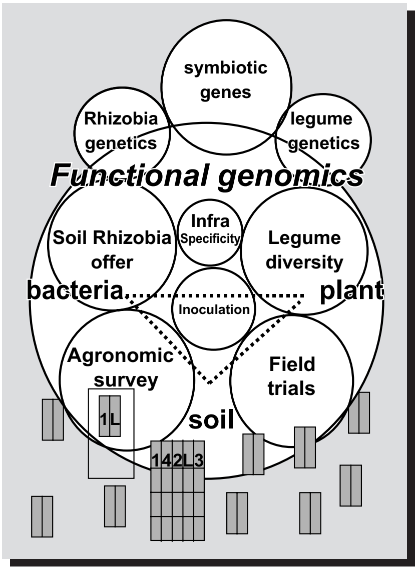

Figure 1. Interdisciplinary cooperation to improve legume symbiotic fixation in the Mediterranean environment.

Nodulation varies in time and space

Nodulation surveys at the flowering stage of common bean in farmers' fields show a large variation in the number and mass of nodules per plant: in Tunisia, no nodules could be detected in Beja and Jendouba, whereas in Bizerte and Cap Bon the mean nodulation varied between 0.1 and 2.3 nodules/plant (21); in Morocco, nodulation was lower in Loukos than in Ain-Atiq (13); in France nodulation varied from less than 5 to more than 50 nodules/plant among 10 farmers' fields over three years in Lauragais (15).

In the fields where N fertilisation increased bean yield, shoot biomass data were plotted as a function of nodulation. Whenever a linear regression was found, the slope of the curve was considered to be an indicator of the rhizobial symbiosis utilisation efficiency, plant N nutrition and growth. This slope varied among sites and fields (15).

Diversity of native rhizobia

Nineteen rhizobia from Morocco and 30 from Tunisia appeared to be at least as efficient as our reference *Rhizobium tropici* CIAT899 with the local common bean cv. Coco, but there was a large variation in their tolerance to salinity in free-living culture (7, 13). The following species of the *Rhizobiaceae* family were found among these isolates: *R. gallicum* and *R. giardini*, proposed as new species by Amarger *et al.* (1); *R. etli* and *R. leguminosarum* bv. *phaseoli* which are commonly found where common bean has been grown for centuries; *Sinorhizobium fredii* and *S. meliloti* found for the first time by Mahmdi *et al.* (24) to be species nodulating common bean in Mediterranean soils; and finally pseudo-*Agrobacterium* (8), confirming the previous isolation of *Agrobacterium* sp. from Acacia sp. nodules by de Lajudie *et al.* (2).

This large diversity agrees with serological studies showing that 62% of the isolates from Tunisia distributed among 19 serogroups (16). In addition the structure of the rhizobial populations differed significantly between the Medjerda Valley, Cap Bon and the semi-arid south of Tunisia.

Selection of symbiotic *P. vulgaris*

Over a three-year period 14 out of more than 100 lines of *P. vulgaris* were selected in Mateur as more productive than the local cv.

Coco (23). Subsequently in a farmer's field in the Medjerda Valley, the lines Flamingo, Ruddy, BRB17, CAN74, DOR585, KID53, SVM29-21 and WAF147 outyielded Coco, with nodulation by native rhizobia as high as 27 nodules/plant, which was significantly higher than 11 nodules/plant in Coco. Although these lines met farmers' demand for pod production, none had the seed characteristics to replace Coco for the white-grain local market. Nevertheless in Lauragais, yield variability was found among 27 white-seeded lines with Diego outyielding the 2.5 t/ha of the local cv. Linex (19). In the glasshouse, P. Rodino (personal communication) found an even larger variation in N_2-dependent growth among 30 landraces from the Iberian Peninsula.

The sensitivity of the above lines to moderate salinity was tested in the glasshouse with N nutrition depending upon either the rhizobial symbiosis with *R. tropici* CIAT899 or urea. Although the nodulation was higher in the glasshouse than in fields, the plant growth limitation by 25 mM NaCl was more with symbiotic than with mineral N, (11). With the rhizobial symbiosis, Flamingo, BAT477, DOR364 and DOR585 were less sensitive than Coco to salinity, whereas Dark, ABA16 and BRB17 were the most sensitive (14).

Selected bean lines with native rhizobia

In order to test whether the above differences in N_2-dependent growth and sensitivity to salinity among common bean lines would express with native rhizobia, cross inoculation trials were performed in the glasshouse with either hydroaeroponics or sand culture. In hydroaeroponics, the reference *R. tropici* was more efficient with Flamingo than with Coco whereas the native *S. fredii* 1a6 was more efficient with Coco than with Flamingo. Under salinity, BAT 477 was superior to Coco with the three rhizobia, by contrast with Flamingo which was inferior to Coco with *S. fredii* but superior with *R. tropici* (10).

In sand, the plant growth was 20% of that in hydroaeroponics and the ranking of symbiotic efficiency was also different (10). No correlation was found between the tolerance to salinity of native rhizobia in free-living culture, and their symbiotic efficiency under moderate salinity.

Nodule function under salinity

Since salt-tolerant symbioses were selected in controlled environments, physiological assays were performed to search for mechanisms involved in the tolerance. The Na content of the nodules was higher in the tolerant BAT477 than in the sensitive Coco. However, it was much lower than the Cl content of the nodules which did not differ significantly between lines (11). Although the higher sensitivity of Coca was associated with higher contents of Na and Cl in leaves, it was concluded from a split-root experiment that the sensitivity of symbiotic common bean to salinity was not due to the toxic effects of Na or Cl accumulation in nodules or leaves (17).

Nitrogenase reduction of N_2 by rhizobial bacteroids in the nodules depends on an intense respiration of the sucrose from the legume photosynthesis. A central role in nodule carbon metabolism is played by phosphoenol pyruvate carboxylase (PEPC) that links bicarbonate with a triose into a C4 organic acid. The latter is involved in the supply of energy to bacteroids, the incorporation of fixed N into amino acids, and the regulation of nodule osmotic pressure and pH. Higher nodule PEPC and malate dehydrogenase were found in NaCl tolerant symbioses (18).

Another enzyme involved in the carboxylation is the carbonic anhydrase that catalyses the synthesis of bicarbonate from CO_2. The nodule-specific carbonic anhydrase, initially found by de la Pena *et al.* (3) in *Medicago sativa* was localised by *in situ* hybridisation on common bean nodule cuts precisely in cells of the cortical parenchyma in between the vascular traces and the infected zone (20). These cells were previously shown by Serraj *et al.* (25) to change their size in soyabean with variation in nodule permeability to the diffusion of O_2, a gas that is toxic to nitrogenase but extremely important for the ATP-dependent reduction of N_2 (5). Nodule permeability was increased by salinity consistent with the hypothesis of its osmoregulation (9), and with over-expression of an MIP Aquaporin in the above cells (22).

Future prospects

FABAMED has established major experimental links between disciplines. The cooperation between bacteriologists and plant breeders has been stimulated by the diversity of *Rhizobiaceae* species in production areas and the specificity of their interactions with selected bean lines. The interactions with physiologists and genomists was facilitated by screening in a controlled environment.

More interdisciplinary work is needed at the field level with agronomists and producers to integrate the improvement of symbiotic nitrogen fixation with yield stabilisation in grain legumes. The link with functional genomics may lead to the identification of candidate genes as tools to further elucidate the mechanisms involved in the efficiency of rhizobial symbiosis and its adaptation to abiotic contraints. This might also help to extrapolate the results obtained with common bean to other grain legumes such as chickpea or faba bean which are important sources of protein for humans in the Mediterranean Basin. ■

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Nitrogen fixation and yield of chickpea in saline Mediterranean zones

Fixation azotée et rendement du pois chiche en zones salines méditerranéennes

by Chedly ABDELLY*, Abdelmajid KROUMA* and Jean-Jacques DREVON**

In many Mediterranean countries, grain legumes with high seed protein content are an attractive choice to meet the huge demand for proteins, both for human consumption and for animal feed. However, it is necessary to enhance their tolerance of biotic and abiotic stresses. Chickpea (*Cicer arietinum*), considered the second most grown legume for human nutrition in the world, is widely distributed in the Mediterranean Basin. In Tunisia, it is the most important food legume. It is cultivated on nearly 35,000 ha, accounting for 33% of the total legume area, but yields never reach 0.7 t/ha (1). In Morocco, chickpea is grown on 77,000 ha but also has low and unstable yields (5). The low level of yields is due to environmental stress (drought, salinity, and mineral deficiencies), a crop management not sufficiently adapted (no fertiliser application) and biotic factors (various diseases and insect pests). Chickpea is one of the most salt sensitive legumes, and it may be useful to screen available germplasm to identify tolerant varieties likely to give higher yields under conditions of moderate salinity. Agronomists, microbiologists, plant breeders, physiologists and molecular biologists from several Mediterranean countries came together in a multidisciplinary research project (FYSAME) to address this need.

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Large genotypic variability in salt response

Significant variations were reported among chickpea lines for efficient nodulation under salt stress conditions. For example, a comparison of the salt responses of five Tunisian chickpea varieties (Amdoun 1, Chettoui, Kasseb, INRAT 88, ILC 482) and one French variety (FV) showed that the growth of Amdoun 1 was stimulated (120% of the untreated control) in the presence of 35 mM NaCl. However, in other varieties growth was significantly inhibited, Chettoui being the most affected, 50% of control (7). Amdoun 1 was also superior in terms of nodule development and growth, and symbiotic nitrogen fixation (SNF). In saline conditions, SNF potentials were at least 3.5 times higher in Amdoun 1 than in Chettoui (Figure 1). Using the same approach, 200 Moroccan lines were screened for nitrogen activity, dry matter, nodule mass, plant colour and total nitrogen under conditions of 0, 25 and 50 mM NaCl in nitrogen-free sandbenches (4.00 m x 2.00 m x 0.20 m) in the greenhouse (5). The inoculum was a mixture of four Moroccan salt-resistant *Rhizobium* strains. Three lines were selected for salt tolerance with respect to SNF and yield. These three Moroccan lines produced 2.1–3.3 t/ha compared with 1.2–1.7 t for control lines and the difference was significant. In the same way, the agronomic survey of chickpea nodulation in saline soils provided a promising approach for the identification of salt tolerant genotypes. For example, genotypes of chickpea selected for nodulation and grain-yielding ability in a saline field over two growing seasons

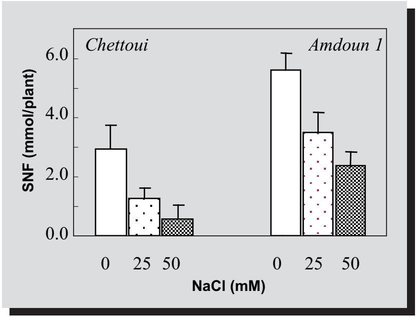

Figure 1. Changes in symbiotic nitrogen fixation (SNF, mmol/plant) with medium salinity in two chickpea varieties. SNF estimated as the difference between N quantities (mmol/plant) at the final harvest and the beginning of salt treatment. Treatment duration is five weeks. Values are means of 10 replicates ± standard deviation.

showed high nodulation and symbiotic nitrogen fixation capacities when grown in pots filled with saline soil (4).

Combining salt-tolerance and nodulation ability

An agronomic survey of chickpea in the main production areas in Tunisia showed that the nodulation on this species varied with site. Nodules were not always observed (1), but when they were present, their number never exceeded 10 per plant and most of them were of small size and apparently ineffective. This suggests that *Mesorhizobium ciceri*, the common chickpea-specific *Rhizobium*, was not present in the sampled soil. It was also reported that bacteria from the *Sinorhizobium* genus are able to establish an effective symbiotic relationship with chickpea. These results suggest also that inoculation with selected strains of rhizobia would be needed if winter chickpea was to be introduced in areas where lack of nodulation is related to a small population of chickpea-specific rhizobia.

Yield potential of chickpea depends on both the associated rhizobia and plant genotype which influence the symbiotic performance and the symbiosis response to environmental stress. Studying the impact of the bacterial partner on the performance of chickpea cv. Chettoui, Mhadhbi *et al.* (3) showed that under unstressed conditions, the *Mesorhizobium ciceri* and *Sinorhizobium medicae* strains seemed to confer to chickpea cv. Chettoui a nodulation, nitrogen-fixing capacity, and shoot dry weight higher than those attained with the *Mesorhizobium mediterraneum* strain. Whatever the associated rhizobia, salt treatment limited significantly the growth and the nodulation of all chickpea plants. Nevertheless, symbioses implicating *M. ciceri* and *S. medicae* strains appeared to be more tolerant to salt than *M. mediterraneum*–Chettoui symbiosis. In the same context, Saxena and Rewari (6), using the classical 'host x genotype interaction' strategy, concluded that chickpea yields can be improved substantially in saline soil by selecting for both a salt tolerant host and an appropriate *Rhizobium* strain. It was subsequently shown in a study of a single host in combination with eight individual strains of rhizobia that the strains most effective in non-saline conditions were also the most effective in salt-affected soil. This suggests that the key feature in sustaining nitrogen fixation under salt stress is the nodulation ability of the host genotype.

Higher rates of SNF under salt stress

The implications of selection for SNF capacity under salt stress for developing new cultivars are two-fold. First, chickpea can be extended as a winter crop to marginal salt-affected areas where few crops can be grown. Secondly, improving SNF can be a successful approach for improving the seed yield of crops in traditional production areas recently affected by salt (5). Exploring genotypic variations in salt response in terms of N_2 fixation in chickpeas also leads to progress towards an understanding of the physiological and molecular SNF mechanisms associated with salt tolerance, and the development of appropriate tools to accompany breeding grain legumes for salt tolerance. For example, comparative studies of sensitive versus tolerant symbioses of chickpea showed that the particular tolerance of the local variety Amdoun 1 seems to depend on its ability to maintain a vigorous leaf area insuring adequate carbon supply for the development of an abundant and efficient nodular system, which in turn determines a favourable rate of nitrogen fixation allowing plants to conserve their growth potential (2). Indeed, plants of Amdoun 1 showed superior ability to protect their leaves and nodules against the overloading of toxic ions, Na^+ and Cl^- (Figure 2). These ions were retained in roots and to a lesser degree in their stems. The superiority of Amdoun 1 is also linked to its capacity to maintain a selective absorption and translocation of K^+ to the leaves, in spite of the abundance of Na^+ in the culture medium. In other studies (3), salt tolerance in some chickpea lines was correlated with their capacity to neutralise reactive oxygen species (ROS) using a complex oxidative defence strategy, particularly in nodules. ROS generation can be enhanced under salt stress and can drastically damage bacteria and plant tissues,

The identification of several chickpea genotypes (Amdoun 1, MCA103, MCA131 and MCA 250) with higher rates of SNF under moderate salinity was a favourable result of the FYSAME initiative (2, 5). ■

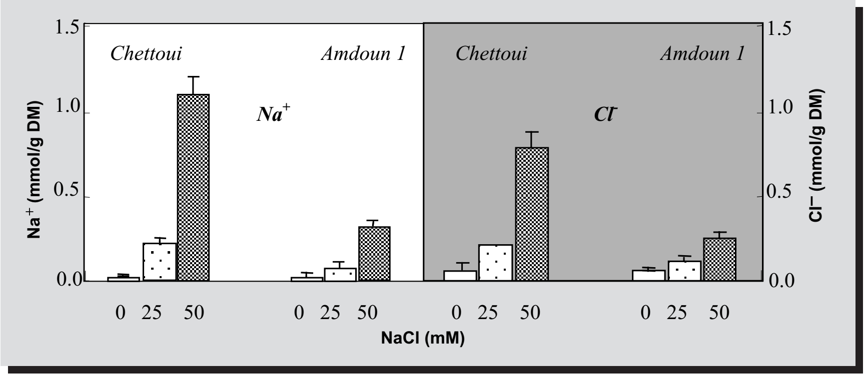

Figure 2. Changes in sodium and chloride concentrations (µmol/g DM) in nodules of two chickpea varieties, with medium salinity. Treatment duration is five weeks. Values are means of 10 replicates ± standard deviation.

Acknowledgements: This work was supported by the FYSAME project No. ERBIC18C960081 of the INCO DC programme of the European Union.

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Insert

"The ability of plants to tolerate salt is determined by multiple biochemical pathways that facilitate retention and/or acquisition of water, protect chloroplast functions, and maintain ion homeostasis. Essential pathways include those that lead to synthesis of osmotically active metabolites, specific proteins, and certain free radical scavenging enzymes that control ion and water flux and support scavenging of oxygen radicals or chaperones. The ability of plants to detoxify radicals under conditions of salt stress is probably the most critical requirement. Many salt-tolerant species accumulate methylated metabolites, which play crucial dual roles as osmoprotectants and as radical scavengers. Their synthesis is correlated with stress-induced enhancement of photorespiration. (…)"

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Osmoregulation in rhizobia: the key role of compatible solutes

La régulation osmotique chez les rhizobia : le rôle essentiel des solutés compatibles

by Daniel LE RUDULIER*

Cellular adaptation to osmotic stress is a cardinal biological process that protects organisms against the lethal effects of dehydratation. Osmoregulation is of great significance in agriculture, since water is the major limiting factor in crop productivity. Salinity is another form of water-related stress which is responsible for major crop losses worldwide. In regions of low rainfall, salts accumulate because percolating moisture is insufficient to wash out salts added by irrigation. In soils subjected to drought or containing an excess of sodium chloride, the water available to the bacteria is restricted, and an efflux of water occurs along the osmotic gradient. This process results in a partial dehydration of the cytoplasm, a phenomenon known as plasmolysis. Such plasmolysis affects the metabolism of the cells and the functions of macromolecules and, ultimately, results in the cessation of growth.

Maintaining internal turgor to survive

To survive and proliferate in environments subjected to fluctuations in osmolarity, soil bacteria must maintain a positive turgor, i.e. a cytoplasmic osmotic pressure higher than that of the extracellular environment. Cellular adaptation to osmotic stress (osmoregulation) is achieved mainly by the modulation of the cytoplasmic osmolarity. The internal turgor pressure which is critical for the expansion of the cell wall during growth is restored, and the initial cytoplasmic volume is regenerated. Such adjustment requires the control of intracellular inorganic ions, and the accumulation of organic compounds in the cytoplasm. These organic solutes, are termed osmolytes or compatible solutes. Most of them can be accumulated to high levels (up to 1–2 M) in the cytoplasm of stressed cells by *de novo* synthesis or transport from the environment. Solutes that alleviate the inhibitory effect of osmotic stress on bacteria when they are added to the growth medium are called osmoprotectants. A typical example is given in Figure 1: addition of glycine betaine in a medium of high osmotic pressure restores the growth of *Sinorhizobium meliloti* (the lucerne-symbiotic *Rhizobium*) which is inhibited by an excess of sodium chloride.

Various accumulated compatible solutes

In general, compatible solutes are polar, highly soluble molecules, and usually do not carry a net electrical charge at physiological pH. These non-toxic low-molecular weight molecules raise the intracellular osmotic pressure, restore the turgor, and protect some macromolecular structures against denaturation. In many bacteria, most compatible solutes are metabolic dead-end molecules not subjected to catabolism or incorporation into macromolecules. However, a remarkable feature of some rhizobia is their ability to use a large range of compatible solutes not only as osmoprotectants but also as carbon and nitrogen sources.

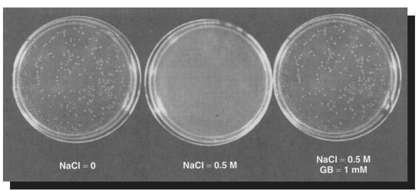

Figure 1. Glycine betaine as an osmoprotectant for *S. meliloti*. The presence of 1 mM glycine betaine (GB) in a medium containing an inhibitory level of salt (0.5 M NaCl) allows normal bacterial growth as in the absence of salt (0 NaCl).

Theoretically, a vast number of compounds could serve the function of osmotic balancing agents in the cytoplasm. Nevertheless, an organism may use only a few compounds to fill this need (5). The prominent osmoprotectants found in rhizobia (Figure 2) are potassium ions, a few amino acids (glutamate), sugars (trehalose, sucrose), polyols (mannitol), quaternary ammonium compounds (glycine betaine, proline betaine, choline and choline-*O*-sulphate), sulphonium compounds (dimethylsulphoniopropionate, DMSP), ectoine, and a small peptide (N-acetylglutaminylglutamine amide, NAGGN).

Betaine transporters, a major role for *S. meliloti*

Under salt-stress conditions, *S. meliloti* can accumulate, through *de novo* biosynthesis, a selection of several compatible solutes (glutamate, NAGGN, trehalose), and their importance varies in response to growth phase and intensity of stress. Moreover, a hierarchy exits among the compatible solutes. Potassium and glutamate serve to regulate the cytoplasmic osmolarity only at relatively low external osmotic pressure, whereas cells subjected to high salt concentrations preferentially accumulate neutral compatible solutes, via uptake systems.

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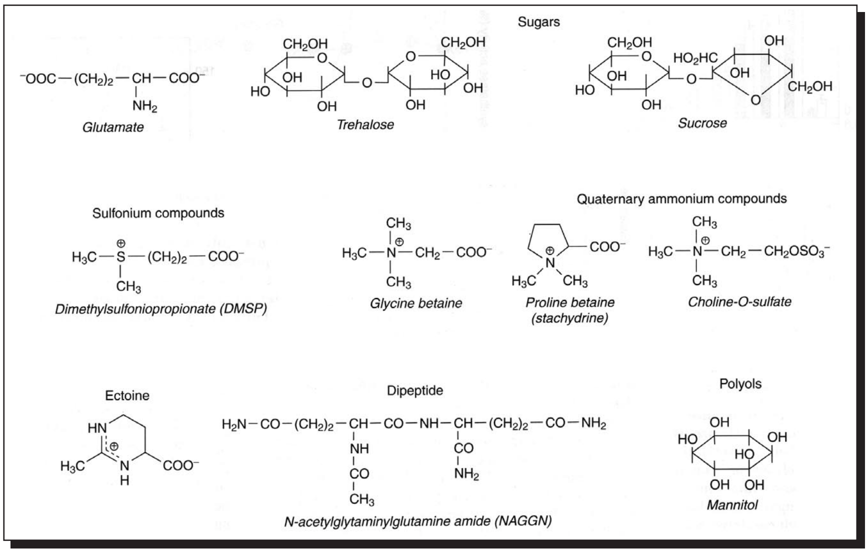

Figure 2. Main compatible solutes in rhizobia.

Betaines are among the most potent osmoprotectants for *S. meliloti*. Glycine betaine can be taken up from the external medium, and two betaine transporters have been fully characterised: the Hut system, an ATP-binding cassette histidine uptake system also involved in low-affinity glycine betaine transport (1), and the BetS system, a betaine choline carnitine transporter (BCCT) required for early osmotic adjustment (2). Recently, we have identified a second ABC transporter (Prb) highly specific for proline betaine, a betaine which occurs widely in *Medicago* species (Alloing, unpublished results). In addition, a choline-ABC transporter (Cho), has been characterised (3). As most bacteria, *S. meliloti* is unable to *de novo* synthesise glycine betaine, and uses a two-step oxidation of choline to produce this compatible solute. Moreover, this bacterium displays the unusual capability to transform choline-*O*-sulphate and phosphorylcholine into choline (6).

Betaine as a source of C and N

A remarkable feature of *S. meliloti* is its ability to use glycinebetaine and proline betaine as carbon and nitrogen sources when grown in a low osmolarity medium or following an osmotic down-shock. The catabolism involves successive demethylations of the quaternary ammonium that generates pyruvate from glycine betaine and proline from proline betaine.

Accumulation versus non-accumulation

The concept of durable compatible solute accumulation within the cells exposed to osmotic stress was established on the basis of studies of enteric bacteria, and several gram-positive bacteria. However, it is not entirely applicable to the rhizobia, particularly to *S. meliloti*. Ectoine which is very osmoprotective against osmotic stress is never accumulated by the cells. Sucrose also acts as a powerful osmoprotectant for *S. meliloti* without being accumulated. These two compounds belong to a class of non-accumulated osmoprotectants, but the mechanism by which they alleviate osmotic growth inhibition remains unknown. Recently, ABC transporters for ectoine/hydroxyectoine (Ehu), and sucrose (Agl) uptake have been identified (4, 7).

Besides *S. meliloti*, the osmoadaptative responses of other rhizobial species have not been thoroughly analysed. ■

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Pea crop behaviour under water deficit

by Jérémie Lecoeur*

At the crop level, the water deficit is a combination of a time of occurrence during the plant cycle, a duration and an intensity. The response of crop to water deficit is as polymorphic as the stress itself, since all the major plant functions (morphogenesis, organogenesis, photosynthesis and transpiration) are affected. That is why, in order to assess the impact of such a stress on yield elaboration, it is necessary to combine a conceptual framework of plant functioning (6) with the assessment of stress occurrence probability (4).

Using the energetic approach to assess biomass production proposed by Monteith (2), three phases have been defined in the pea cycle: (i) setting-up of above- and below-ground captors (from germination to the beginning of flowering), the period when the foliar area and its maximal radiation interception efficiency are defined, (ii) accumulation of biomass (between the beginning of flowering and the end of the seed abortion limit stage) when the main factor is the plant ability to transform radiation interception into biomass (radiation use efficiency), (iii) remobilisation for grain filling (between the end of the seed abortion limit stage and the plant physiological maturity).

The early phase of flowering is critical

Except in the case of early and severe water deficit, which is rare in most European cultivation areas, most events in the first phase are only slightly affected. However the final foliar area is frequently reduced (7): there is no reduction in the radiation use efficiency at the beginning of flowering but there is a rapid decrease at the end of the cycle (5).

On the contrary, the radiation use efficiency is frequently reduced significantly between the beginning of flowering up to the end of the seed abortion limit stage, which leads to a rapid decrease in the rate of biomass accumulation and seed number per plant (3). This corresponds with the critical phase for a pea crop facing water deficit: high probability of moisture stress and high crop sensitivity since seed number is a major component of crop yield (1). This is why irrigation is strongly advised during this period. In the third phase there is a high probability of water deficit but there is a retro-control between the biomass production and the number of grains during seed filling, therefore the seed weight is not strongly affected in most of the cases.

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SPECIAL REPORT
DROUGHT AND SALINE STRESS

AQUARHIZ: Modulation of plant-bacteria interactions to enhance tolerance to water deficit for grain legumes in the Mediterranean dry lands

by Carmen VARGAS*

The primary goal of AQUARHIZ[1] is to increase the production of chickpea, common bean and faba bean in Algeria, Egypt, Morocco and Tunisia, where their nutrition and yield are affected by water deficit. The project is founded on the preceding evidence for inter- and intra-specific genetic variability of legumes for tolerance to water deficit, and in the demonstrated capacity of specific rhizobia to enhance the tolerance to water deficit by the plant.

The project involves 10 Participants from 7 countries: 3 EU States (Germany, France and Spain) and 4 DC States of the Mediterranean basin (Algeria, Egypt, Morocco and Tunisia), in which grain legumes are omnipresent as food products, both for human and animal consumption.

Assessing symbiosis combinations

Aquarhiz research is based upon a starting collection of lines of chickpea, common bean, and faba bean tolerant to abiotic (salinity, iron or phosphorous deficiency) or biotic (bacteria, viruses, fungi or nematodes) constraints, and a number of native rhizobial strains isolated from drought- and/or salt-affected, whose symbiotic combinations have been tested in glasshouses under water-deficit conditions. In parallel, local strains have been isolated, identified, and included in the above screening. In addition, survival of selected strains in inoculant formulations has been analysed, and molecular tools have been developed and used to monitor these strains after field release when used as inoculants.

Investigating tolerance processes

Basic research is also performed in order to elucidate the mechanisms of tolerance to water deficit of selected legume varieties and strains with the aim of identifying genes that could be used for marker-assisted selection in breeding programmes. This year activities have been focused on (i) screening the variability in response to salinity among common bean and chickpea RILs and lines, (ii) different aspects of nodule functioning under water deficit, (iii) transcriptome analysis of contrasting lines of common bean and chickpea, (iv) the expression of key genes in nodules of contrasting lines, and (v) the physiological and molecular characterization of the osmoprotection mechanisms of salt- and/or drought tolerant strains.

Assessing progress with farmers

On farm trials are being conducted in the four southern Mediterranean partner countries, in a series of reference regions. Most of these trials, which involve the use of selected strains as inoculants for stress-tolerant lines of common bean, chickpea, and faba bean, are now on-going and some preliminary data indicate an enhancement of plant growth and grain and pod yield by inoculation, although differences are observed between analysed sites. In parallel to the field experiments, an economic analysis is being performed to assess the feasibility of the proposed strategy.

In order to establish a local partnership for the exploitation of results, a Local Multi-Stakeholder Panel (LMSP) is being set up in some reference regions of Algeria, Egypt, Morocco, and Tunisia. This will enable the project partners to interact with farmers, local extension services, inoculants industrialists and local policy-makers, and discuss the knowledge gathered on grain legumes from previous and on-going projects for the benefit of the region. ■

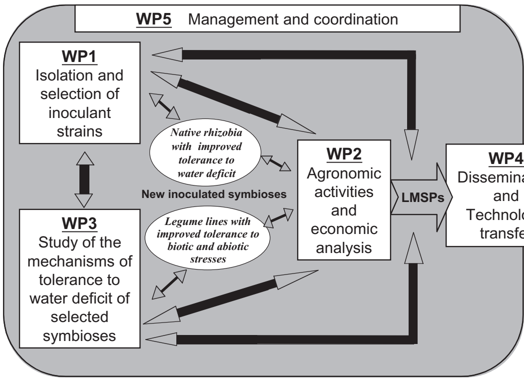

Figure 1. Structure of AQUARHIZ ativities.

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[1] INCO-CT-2004-509115, www.grainlegumes.com/aquarhiz

CGIAR Generation Challenge Programme

The Challenge Programme aims to use advances in molecular biology and harness the rich global stocks of crop genetic resources to create and provide a new generation of plants that meet the needs of resource-poor farmers.

Drought tolerance is one area of interest for the Programme. The recent revolutions of genomics and bioinformatics offer real opportunities for dissecting drought tolerance into component traits and then developing tools to manipulate the underlying genes. Reconstructing effective drought traits will also require considerable advances in whole-plant physiology modelling to achieve a global impact.

The Programme is divided into five sub-programmes:

1. *Genetic Diversity of Global Genetic Resources*
Jean-Christophe Glaszmann (glaszmann@cirad.fr)
2. *Comparative Genomics for Gene Discovery*
Hei Leung (h.leung@cgiar.org)
3. *Trait Capture for Crop Improvement*
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4. *Genetic Resources, Genomic, and Crop Information Systems*
Theo van Hintum (theo.vanhintum@wur.nl)
5. *Capacity Building*
Carmen De Vicente (c.devicente@cgiar.org)

Source: http://www.generationcp.org/index.php

Analysis of the response to abiotic stress in legumes

Analyse de la réponse aux stresses abiotiques chez les légumineuses

by Martin CRESPI*

Environmental fluctuations such as soil salinity, cold and drought stress represent a major constraint on agricultural productivity. Water availability is the major factor controlling the distribution of vegetation over the earth's surface. In fact, crop yields are more dependent on an adequate supply of water than on any other single factor, and environmental stress (cold, drought and salt) represents the primary cause of crop losses reducing average yields by up to 50% (2). Even plants with an optimum water supply experience transient water shortage periods when water absorption cannot compensate for water loss by transpiration, a situation that depends largely on environmental factors such as temperature, relative humidity and wind speed. Many other environmental stress factors, such as cold, salt and high temperature, have a water-stress component. In addition, about 40% of the land surface of the world can be categorised as having potential salinity problems. It is interesting to note that varieties of single plant species exhibit a high degree of variation in salt and drought tolerance suggesting that only a few key genes might enhance plant adaptation to adverse growth conditions (1).

Legumes for improving soil fertility

Due to their capacity to grow on nitrogen-poor soils, legumes are interesting candidates for improving saline soil fertility and helping to reintroduce agriculture to these lands (4). In contrast to *Arabidopsis* and the majority of other crops, legumes are very important both ecologically and agriculturally because of their ability to fix nitrogen in the root nodules in a symbiotic interaction with soil rhizobia. However, legumes, like most plants, are very sensitive to salt levels and soil drought status, experiencing water deficit due to osmotic stress possibly coupled with biochemical perturbations induced by the influx of sodium ions. For example, abiotic stress factors are the major constraints to chickpea production and losses of up to 30% have been estimated to be due to drought and cold stress (estimated from ICRISAT calculations). The physiological consequences of environmental stress in legumes depend on the specific characteristics of these plants. Accumulation of nitrogen in seeds depends largely on efficient nitrogen fixation in the root nodules, a sensitive target for abiotic stress. Reductions in photosynthate production due to environmental stress rapidly influence the function of the root nodule, shoot and root growth as well as the process of grain filling. Possible approaches to improve crop productivity under adverse environmental conditions require a better understanding of the mechanisms involved in the response to abiotic stress. Genes induced specifically during environmental stress may serve to adapt plant cells and tissues to this restrictive condition.

Gene regulation, the key to plant tolerance

Understanding the pattern of activation of gene expression during environmental stress will lead to improved knowledge of the interrelationships of the multiple signalling systems that control stress-adaptive responses in legumes. Induced genes are good candidates to be involved in osmotolerant responses in plants, particularly those putative regulatory genes such as transcription factors or protein kinases as has been shown in other organisms (8). Indeed, increased levels of stress tolerance can be engineered into plants by reprogramming the expression of endogenous regulatory genes. Stress-inducible transcription factors, which regulate the expression of many other genes, provide a view of the complexity of plant signalling transduction pathways involved in the adaptation of plants to stress conditions (3). In addition, several biochemical responses have been involved in stress adaptation such as the synthesis of osmoprotectants and antioxidant responses to control cell damage due to salt stress (1). The linkage of transcriptional and biochemical responses with regulatory mechanisms involving quantitative trait loci (QTLs) associated with tolerant cultivars may be crucial to determine the relevance of these genes to tolerance traits.

The advent of genomic approaches allowed changes induced by abiotic stresses on a global scale to be analysed at the level of the whole organism. In *Arabidopsis*, analysis of the transcriptome changes occurring during cold, drought and salt stress in a survey of 7,000 genes showed a shared response for a majority of cold and drought stress-regulated genes, supporting the hypothesis that a common set of signal transduction pathways are triggered during different stress responses (6). Around 11% of the stress-inducible genes are potential transcription factors further confirming the relevance of gene regulation in stress adaptation.

Continued overleaf…

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… continued from previous page

Which genes are induced by stress in legumes?

Hence, the induction of gene expression by environmental stress must be exploited to unravel mechanisms dealing with abiotic stress tolerance in the agriculturally important grain legumes. The *Arabidopsis* model is likely to be very different from legumes in the responses to stress in relation to grain filling, nitrogen utilisation, fixation and transport, root architecture and interactions – all physiological processes that are fundamentally different in legumes. Hence, the usefulness of developing a legume model has become increasingly relevant in recent years. Significant progress is being made at the genetic and genomic levels using the model legume *M. truncatula*, such as macro- and microarray analysis, reverse genetics, genome sequencing and other high throughput techniques (7, 5). In addition, in several legumes such as chickpea and *M. truncatula*, sensitive and tolerant varieties have been characterised although very little is known about the genes involved in these responses. The characterisation of genes involved in the differential behaviour of these cultivars may constitute a good basis from which to extrapolate these results to other grain legumes.

First set of genes tracked down with omics

In the context of the Grain Legume Integrated Project[1] several laboratories are characterising the physiological changes occurring during environmental stress at the level of the transcriptome and the metabolome in *M. truncatula*. For example, around 380 genes have been identified as induced by stress responses in the model legume of which 75 seem to be common between drought stress in pea and salt stress in *M. truncatula*. Gene regulatory networks and changes in metabolic fluxes occurring in pea and chickpea are being explored now to extrapolate these results. Interestingly at least 10% of these genes seem to play regulatory roles and the potential role of critical common regulators of gene expression and metabolic adaptive responses is now being attempted using functional reverse genetics approaches in *M. truncatula* (e.g. TILLING, deletion mutagenesis and transgenic RNA interference approaches). These techniques will allow the generation of *Medicago* plants affected by the functioning of selected genes. This analysis is being coupled to the characterisation of QTLs linked to abiotic stress tolerance at the genetic level in *M. truncatula* and chickpea in order to evaluate the control mechanisms exerted by the QTLs on gene expression patterns. Dissection of the regulatory networks affected by environmental stress factors will lead to the production of a cDNA microarray carrying diagnostic, stress–responsive cDNAs from the Galegoid section of the legumes to ensure transmission of the results to the legume breeding industry. This will serve to define a rational approach for grain legume improvement. ■

[1]In the GRAIN LEGUMES IP (FOOD-CT-2004-506223), the sub-part 'Abiotic stress" aims to analyse the regulatory networks affected by environmental stresses during pod setting and grain filling:

- identification of pea and chickpea genes which are homologous to known stress-related genes of *M. truncatula* and study of their expression during environmental stress;
- setting up and screening of three SSH cDNA libraries for chickpea and *M. truncatula*;
- refining expression patterns from potential regulatory genes (dedicated macroarrays);
- identification of QTLs associated with stress tolerance.

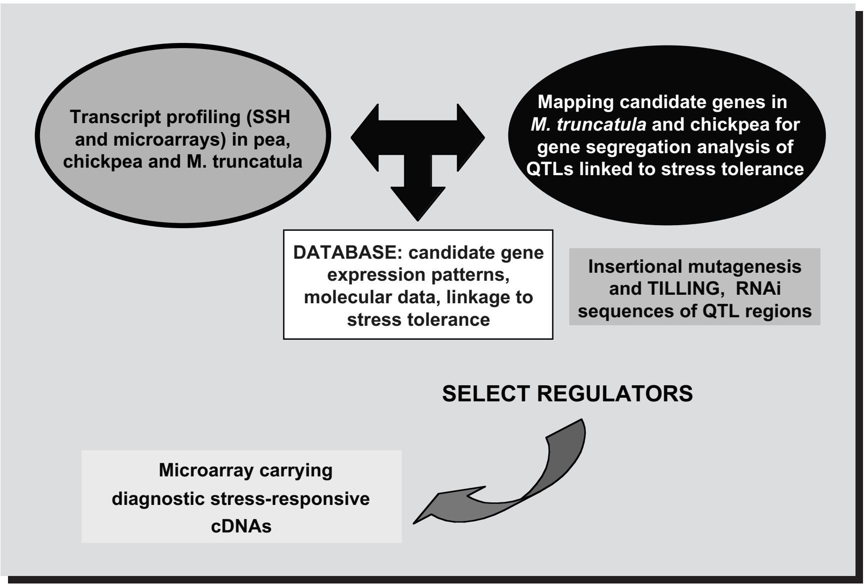

Figure 1. Scheme showing the interaction between transcript profiling and QTL characterisation for the establishment of a legume database for abiotic stress responses. Transcript profiling for diverse species will identify group of genes showing differential expression patterns between sensitive and resistant cultivars. Co-localisation of these genes with known QTLs involved in abiotic stress tolerance may help to identify selected regulatory genes involved in these responses. Through reverse genetic approaches, we plan to analyse the role of these regulators. Selection of common critical regulators may serve for the construction of a diagnostic micro-array to characterise stress responses in legumes.

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Grain legumes in the current CAP reform

Les légumineuses à graines et la réforme actuelle de la PAC

by Anne SCHNEIDER*, Jean-Paul LACAMPAGNE** and Gaetan DUBOIS*** on behalf of GL-PRO[1]

A reform of the Common Agricultural Policy (CAP) that changes the way the EU supports its farm sector, was adopted by the EU farm ministers on 26 June 2003[2]. The legal texts were adopted formally at the Agriculture Council of September 2003.

New CAP reform means a single payment

The core principles of the new CAP are:

– *the full decoupling within the single payment scheme*: the farm subvention is independent of the volume of crop and animal production (allowing farmers' decisions to be market-driven); it is based on an average of the previous payments between 2000 and 2002 in each reference region;

– *modulation*: a reduction in the direct payments for bigger farms (if over €5000/year) by 3% in 2005, 4% in 2005 and 5% in 2006 and in the following years up to 2013; this will finance additional rural development measures;

– *cross-compliance*: any aid is linked to environmental, food safety, animal and plant health and animal welfare standards.

Partial decoupling and product specificities

The EU Member States may decide to choose only a partial decoupling of the payment (either at national or regional level) and therefore maintain a proportion of direct aid to farmers in its existing form ('re-coupling'), notably where they believe that there may be a disturbance to agricultural markets or to avoid the abandonment of production. The re-coupling could concern up to 25% of the arable crop component of the single payment, which is then used for specific crop aid on the basis of the previous CAP rules. Specific payments (so called 'top-up' payments) could also be granted to support some agricultural activities for environmental or quality reasons.

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The consequences for grain legumes vary according to the species:

– for pea, faba bean, lupins (the so-called 'protein crops' in the EU regulation): a specific payment of €55.57/ha, with a Maximum Guaranteed Area of 1.6 million ha (for EU-25) since 2004[3];

– for chickpeas, lentils and vetches (the so-called 'grain legumes' in the EU regulation): integration into the single payment scheme from 2006.

Current transitional situation

The different elements of the new CAP are being introduced between 2004 and 2007, depending on the decisions of individual countries. The single farm payment will come into force in 2005, but a Member State that needs a transitional period due to its specific agricultural conditions may apply the single farm payment from 2007 at the latest.

The decoupled payment (all or part of the single payment) can be different from one country to another since it can be based on either:

– the amount of payments received in the past (standard model or historical model); or

– the area of farmed land (regional model with a single payment per ha of agricultural land); or

– a combination of the two models are possible (hybrid model).

In France and Spain this will be calculated from the average of the previous payments between 2000 and 2002 (standard model). Denmark and Germany have adopted a hybrid model from 2005 to 2009 and from 2010 to 2013 there will be a gradual transition to the pure regional model (i.e. a regionally uniform payment).

For grain legume species, the new scheme has been available since 2004, but the transitional period varies according to countries and species (see Table 1). For peas, faba beans and lupins harvested in 2005, France and Spain will use the same systems as 2004, whereas Denmark and Germany will apply the new system from 2005.

For chickpeas, lentils and vetches, the standard payment in 2005 is €181/ha but with a total payment ceiling in some countries (i.e. €1,331,000 in France, €60,518,000 in Spain and €2,100,000 in Greece). ■

[1]GL-Pro (2003–2006) European extension network for the development of grain legume production in the EU. Further information from Gaétan Dubois (g.dubois@prolea.com) and http://www.grainlegumes.com/gl-pro/

[2]More at http://europa.eu.int/comm/agriculture/capreform/

[3]In addition, since 2001 harvest, peas, faba beans and lupins (sweet or bitter) can be grown in the 'non-food set aside areas' and benefit from the non-food-fallow payment (€/ha) of €63/t multiplied by the reference yield (t/ha). This is sometimes used for green manure (or might be used for energy purposes).

Table 1. Payments for EU farms.

	Transitional period	New CAP	
	Harvests 2004 and 2005 in France and Spain *Harvest 2004 in Germany and Denmark*	*From 2005 in Germany and Denmark*	*From 2006 in France and Spain*
Arable crops	(63 x RY) €/ha + €55.57/ha (*) of specific payment (for protein) for pea, faba beans and sweet lupins	A + €55.57/ha (*) of specific payment for pea, faba beans and sweet lupins	(0.75 x A) + [0.25 x (63 x RY)] + €55.57/ha (*) of specific payment for pea, faba beans and sweet lupins
Chickpeas, lentils, vetches	€181/ha (**) for chickpea, lentil and vetches		

RY = the regional reference yield (t/ha); A = single farm payment; (*) within the limit of the Maximum Guaranteed Area of 1.6 million ha for the EU-25 (not exceeded in 2004); (**) within the limit of a total payment ceiling in some countries (i.e. €1,331,000 in France, €60,518,000 in Spain and €2,100,000 in Greece) for 2005. Between 2000 and 2003, the vetch payment has been reduced regularly because it has exceeded the ceiling.
Nota bene: For cereals, there is also an additional premium for quality of durum wheat. In addition, there is an intervention price for some cereals (€101.31/t).

Grain legumes recommended in the UK

Les pois et féveroles recommendés en Angleterre

by Simon KIGHTLEY*

Far from regarding peas and faba beans as low value protein crops for livestock, the majority of British growers target the higher value human consumption and pet food markets. This is reflected in the seed certification statistics for 2004, where the areas entered for certification broke down into 54% large blue peas, 35% marrowfat peas and only 10% of the generally higher yielding white peas. This addresses the high value of the pet food industry, in the case of the large blues and human consumption markets for marrowfat peas. Spring faba bean production is strongly skewed towards types that have good characteristics for human consumption in the Middle Eastern and North African markets, namely, pale smooth skins and a white hilum. Of this type, crop areas entered for certification made up 84% of the total. Here again, market preference is often at the expense of higher yielding, non-quality varieties.

The NIAB/PGRO Recommended Lists for 2005[1] show major improvements in yields from new varieties of both white and large blue peas but reflect a period of consolidation for marrowfat peas and faba beans. Tables 1 and 2 summarise the most important attributes of the recommended varieties. Varieties are considered for recommendation after two years of National List trials, paid for by breeders and a third year of Recommended List trials paid for by a levy on seed sales which is administered by the Processors and Growers Research Organisation (PGRO).

Table 1. Peas: extract from NIAB/PGRO Recommended List.

Variety	Type	Relative yield (%) Control mean = 5.15 t/ha	Standing ability (9 = good,1 = poor)	Resistance to downy mildew (1 = poor, 9 = good)	Year first listed
Bilbo New	W	111	7	6	2005
Alezan New	W	109	5	6	2005
Beetle	W	107	5	7	2004
Sioux New	W	107	7	6	2005
Enigma	W	104	6	7	2004
Arrow	W	98	5	7	2000
Cooper New	LB	108	7	6	2005
Venture	LB	99	6	8	2002
Nitouche	LB	97	7	7	1999
Kahuna	MF	93	5	5	2004
Orka New	MF	92	4	5	2005
Princess	MF	91	5	3	1987
Samson	MF	91	3	6	2001
Maro	MF	85	2	1	1985

Types: W – white, LB – large blue, MF – marrowfat

Table 2. Spring faba beans: extract from NIAB/PGRO Recommended List.

Variety	Type	Relative yield (%) Control mean = 4.19 t/ha	Standing ability (9 = good,1 = poor)	Resistance to downy mildew (1 = poor, 9 = good)	Year first listed
Fuego New	W	112	8	7	2005
Ben New	W	104	8	5	2005
Hobbit	B	104	8	8	2003
Meli	B	104	7	6	2000
Syncro	W	103	8	7	2003
Quattro	W	102	8	5	1998
Compass	W	100	8	7	2001
Nile	W	98	8	5	2003
Victor	W	92	8	3	1989
Maris Bead*	B	84	6	8	1964

Type: W – white hilum, B – black hilum.
*Maris Bead is a small grain variety grown for pigeon feed.

Peas

Bilbo and Sioux are the first varieties with good standing ability *and* high yield, to be recommended for several years and they could stimulate new interest in their crop sector. Alezan's additional strength is good downy mildew resistance, which should offer savings on fungicide seed dressings. The large blue pea, Cooper, will cause considerable excitement as a replacement for the current market leader, Nitouche (38% certification area in 2004). Nitouche became very popular because of its good standing ability and suitability for the micronising market, with good colour retention and large grain size. Now Cooper appears to promise a 14% yield improvement with the same good standing ability and grain quality but slightly poorer downy mildew resistance. The new marrowfat pea, Orka, has yield and general characteristics similar to others in its group. Its success will depend largely upon its processing quality (soaking and shape retention after cooking) and this is often not clear until commercial crops have been grown over a range of weather conditions in the first few seasons. It should be noted that the continuing success of the normal-leaf type Maro is because of its quality characters despite the handicaps of low yield and very poor field and disease characters.

Spring faba beans

For several years the spring faba bean market leader has been Victor which is noted for its good grain appearance and thin skin which has advantages in cooking. Its low yield and susceptibility to mildew are making it increasingly uncompetitive and Syncro, Compass and Nile are eroding its market share. Fuego presents a major yield improvement and, with good standing ability and downy mildew resistance, it looks very promising. However, in samples seen so far, its overall grain appearance is less attractive than some of the white-hilum varieties and this may limit its export potential. Ben is marginally higher yielding than the previous best white-hilum type but also may lack the absolute grain quality to impress the buyers.

Improvements in yield, quality, field characters and disease resistance are absolutely imperative in grain legumes as we move into an era of farm payments which are de-coupled from crop production and, in the UK, the talk is increasingly of wheat/wheat/rape rotations. The evidence of this year's Recommended List revisions suggest that this can and is happening. ■

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[1]For further information and full details of NIAB/PGRO Recommended Lists contact www.niab.com or www.pgro.co.uk or simon.kightley@niab.com.

Development of internationally accepted methods for measuring pulse quality

Définir des procédés internationalement reconnus pour mesurer la qualité des graines de légumineuses

by Ning WANG*

The mature grains of grain legumes are marketed as dry products usually called pulses in the trade and industry sectors. They are exploited primarily for their rich protein content. Pulse is a derivative of the Latin word *puls*, meaning 'porridge'(1). In some areas of the world, pulses are also known as grain legumes. Production and international trade in pulse crops, particularly dry peas, lentils, chickpeas and beans, has increased dramatically over the past 15 years. The principle marketing countries have been Australia, Canada, the United States of America and the European Union. Pulses provide protein, fibre, complex carbohydrates, vitamins, and minerals to the diet and are an important part of vegetarian diets and an increasingly important component in animal feeds. While standards for nomenclature and quality measurement for pulses exist between some buyers and sellers of pulses, these do not have universal recognition. It is possible that in as many as 30% of trades involving pulses, differences in methodology and nomenclature between buyers and sellers may result in disputes requiring arbitration. This suggests a critical need for developing standard methods for testing the quality characteristics of pulses and a common international language for trade. Providing more definitive quality assessment methods would reduce trade disputes.

Table 1. Quality parameters identified by the International Pulse Quality Committee (IPQC).

Seed size	Moisture
Water absorption	Crude protein
Split yield & dehulling efficiency	Fibre
Cooking quality & time	Starch
Canning quality	Trypsin inhibitor activity (TIA)
Seed coat integrity	Vicine–convicine
	Tannins

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International Pulse Quality Committee (IPQC)

The International Pulse Quality Committee (IPQC) was created in 2000 with a vision to facilitate the marketing of pulses through the development of standardised nomenclature and methods of testing quality parameters in order to meet customer needs. Members of the IPQC include Canada, the United States, Australia and Europe, representing the scientific community, and growers and traders of the world's leading pulse exporting countries.

One goal of the IPQC was to assign nomenclature for each type and class of pulse crop to end the confusion that exists due to the multitude of names currently in use. In addition, there were very few current internationally accepted methods for evaluating pulse quality. The IPQC identified 13 quality parameters aimed at creating common measurements for everything from seed size, moisture, and crude protein, to dehulling efficiency, and cooking and canning qualities (Table 1). The type of work required ranges from complete development of new methods, to verification and development of internationally recognised precision and accuracy for established methods.

AACC Pulse and Grain Legume Technical Committee (TC)

The American Association of Cereal Chemists (AACC) is a non-profit international organisation of nearly 4,000 members who are specialists in the use of cereal grains in foods. AACC has been an innovative leader in gathering and disseminating scientific and technical information to professionals in the grain-based foods industry worldwide for over 85 years. AACC has modified its mandate to include pulses and was very interested in developing a committee to promote pulse-oriented methodology. In 2002, an AACC Pulse and Grain Legume Technical Committee (TC) was established. One of the goals for the Pulse and Grain Legume TC is to act on developing new methods and revising existing methods for pulses. Another goal is to organise technical sessions on pulses and grain legumes at the AACC annual meetings. Methods developed by the IPQC will be tested and verified by the AACC Pulse and Grain Legume TC to ensure that the methods are valid internationally. The AACC will also publish the methods so that they are accredited and available for labs around the world.

Standardised names and seed testing

The IPQC is creating a standardised naming system (nomenclature) for pulse crops. While local names may vary from region to region, the pulse industry will have an international standardised system that can be used for each type of pulse crop, based on seed shape and colour, seed coat characteristics, and the colour of split seed. These internationally recognised names for each of the major pulse crops will be available on the Internet and this international naming system will help all countries communicate for trade.

(Ning Wang, CGC, Winnipeg, Canada)

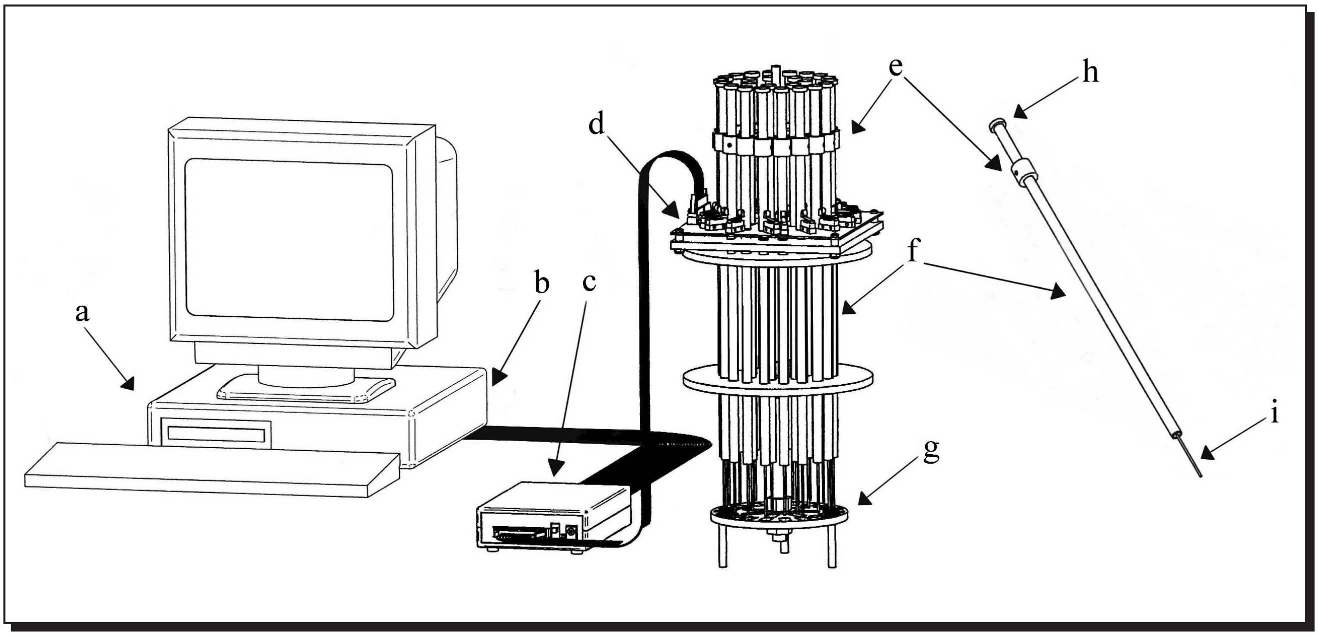

Figure 1. A schematic representation of the automated Mattson cooker (a-PC; b-I/O card; c-Interface box; d-Circuit board assembly; e-Actuator; f-Plunger; g-Mattson cooker; h-Plunger cap; i-Stainless steel probe).

There has been good progress on developing standard testing methods for evaluating pulse quality. Common testing methods for measuring quality will help define the characteristics that each country requires in its pulses.

Pulse crops contain moisture that must be estimated in order to apply the appropriate premium or discount when trading these crops. However, there was no published international standard method for the determination of moisture content in pulses. Through collaboration with researchers from Canada, the United States and Australia, a gravimetric procedure for determining the moisture content of pulses has been developed and subjected to an interlaboratory study. The method has been accepted and published by the AACC as a standard reference method (2).

Water hydration capacity of pulses is another important quality parameter and is defined as the amount of water that whole seeds absorb after soaking in excess water for 16 h at room temperature (22 ± 2 °C). Pulses are generally soaked before cooking to ensure uniform expansion of the seed coat and cotyledon for uniform cooking and to ensure their tenderness. The ability of seeds to hydrate has been linked to cooking or canning quality. Several methods for measuring the water hydration capacity of pulses have been reported, but no universally accepted methods exist. A collaborative study on a method for determining the water hydration capacity of pulses was carried out in the summer of 2004 and the precision of the method was established. The AACC Pulse and Grain Legume TC has approved the method.

A method for determining the crude protein content of pulses and other agricultural commodities has been developed and is at the Draft International Standard stage in ISO (the International Organization for Standardization).

Cooking time is one of the main considerations used for evaluating pulse cooking quality. Longer cooking times result in a loss of nutrients and could limit end uses. Several methods for measuring the cooking time of pulses have been reported. One method for measuring cooking time is to evaluate tenderness using a sensory panel. Another method is a tactile method described by Vindiola *et al.* (3). Both the sensory panel and tactile methods are subjective and time-consuming. A method for determining the cooking times of pulses using an automated Mattson cooker apparatus (Figure 1) has been developed (4) and this method was more objective, much easier to carry out and more resource efficient compared with the sensory panel and tactile methods. A collaborative study will be needed to establish the precision of the method.

A method for measuring size of pulses using an Image Analysis Technique has been developed, which could be applied to lentils, peas and chickpeas and, after further development, to navy beans (*Phaseolus vulgaris*) (5). An inter-laboratory study is needed to verify the method.

The methods for evaluating dehulling quality, as well as the firmness of cooked pulses, are mostly completed and are near the interlaboratory test stage.

A collaborative effort is required to accomplish the tasks identified by the IPQC (Table 1). Researchers who work with pulses/legumes or are interested in related research are welcome to participate in the collaborative studies organised by the IPQC (co-chaired by Dr Joe Panozzo at Joe.Panozzo@dpi.vic.gov.au and Dr Albert Vandeberg at vandenberg@usask.ca) and AACC Pulse and Grain Legume Technical Committee (TC) (chaired by Dr Ning Wang at nwang@grainscanada.gc.ca). ■

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GRAIN LEGUMES

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Study of water deficit in pea (Photo J. Lecoeur, ENSAM, France)

PHOTO DE COUVERTURE:
Etude du stress hydrique chez le pois (Photo J. Lecoeur, ENSAM, France)

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Special report

Lupins for human consumption and health

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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.

CEBECO SEEDS

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The activities of Cebeco Seeds are: breeding, production and marketing of the full range of agricultural seeds including forage and amenity grasses. Emphasis on cereals, pulses, flax and grass seed.

UFOP

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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.

Pulse Canada

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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.

To be added to this sponsorship list, do not hesitate to contact the AEP Office.