



# LEGUME PERSPECTIVES



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**Merging knowledge on diverse legume research topics**

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**T**he issue of our journal *Legume Perspectives* you are (virtually, though) hold in your hands was designed as another proof that one of the fundamental aims of the International Legume Society (ILS) has already been brought into reality: to gather together the basic and applied research communities dealing with various topics on soybean, grain legumes, forage legumes and other groups of legumes, demonstrating how they may successfully assist and fully complement each other, saving more and more precious time and limited resources. Similarly to the issue 6, this one will surely refresh your memories of the ILS first meeting, held in Novi Sad in May 2013, not only of its scientific character, but also of the events of other kind, and hopefully may bring you novel ideas to be conceived, developed and presented at the forthcoming second ILS meeting, scheduled for October 2016 in Portugal. Enjoy your reading and may it be both interesting and useful!

**Aleksandar Mikić and  
Vuk Đorđević**

Managing Editors of  
*Legume Perspectives* Issue 8

## CARTE BLANCHE

- 4 Noel Ellis, Julie Hofer: Sgt. Legume Science Joint Club Band

## RESEARCH

- 5 Marina Tomičić, Vuk Đorđević, Jegor Miladinović, Ljiljana Brbaklić, Sanja Mikić, Dragana Trkulja: Hitchhiking mapping in soybean: Perspectives for more targeted future breeding
- 7 Beat Boller, Franz Schubiger, Peter Tanner: Breeding a red clover variety for grazing use
- 9 Ozlem Onal Asci, Fatih Nalbanto, Metin Deveci, Zeki Acar: Hay quality of Persian clover (*Trifolium resupinatum* var. *resupinatum*) affected by soil properties
- 12 Karine Gallardo, Isabelle d'Erfurth, Christine Le Signor, Vanessa Vernoud, Grégoire Aubert, Julia Buitink, Jérôme Gouzy, Jean-Marie Prosperi, Judith Burstin, Richard D. Thompson: Use of translational genomics to identify genes important for legume seed development
- 15 Sara Fondevilla: The use of next-generation sequencing technologies in legumes
- 17 Nuno F. Almeida, Diego Rubiales, M. Carlota Vaz Patto: Transcriptional profiling comparison of related grain legume genes differentially regulated in response to infection with *Ascochyta* spp.
- 19 Johann Vollmann, Daisuke Watanabe, Martin Pachner, Jonibek Khudaykulov, Tomáš Ložák: Soybean quality: Adaptation to European needs
- 21 Pietro P. M. Iannetta, Euan K. James, Cathy Hawes, Laura Lopez Del Egado, Alison Karley, Oluyinka Oluksosi, Jos Houdijk, Viv Crampton, Martin Moench, Gabriele Palomba, Jacopo Ianieri, Fergus Clark, Ken Duncan, Geoff R. Squire, Gavin Ramsay, Graeme Walker: Enhancing the economic potential of beans using *Vicia faba* L.: Crop performance and the use of air-classified grain components in animal feeds and brewing
- 24 Hannu Känkänen: Reducing use of fossil energy by biological N fixation
- 26 Aleksandar Mikić: All legumes are beautiful, but some legumes are more beautiful than others
- 28 Alex De Vlieghe, Gerda Cnops: Lucerne and clover in pure stand or in association with ryegrasses under intensive conditions in Flanders
- 31 Ana Marjanović-Jeromela, Aleksandar Mikić, Sreten Terzić, Svetlana Vujić, Vojislav Mihailović, Branko Čupina, Pero Erić, Đorđe Krstić, Aleksandra Dimitrijević, Ivana Imerovski, Dragana Miladinović: Intercropping annual legumes and brassicas for forage production
- 33 Birte Boelt: Seed production of white and red clover
- 35 Diego Rubiales: *Ascochyta* blight resistance in cool season food legumes
- 37 Stefano Pavan, Angelo Raffaele Marcotrigiano, Nicoletta Bardaro, Valentina Bracuto, Francesca Ricciardi, Concetta Lotti, Luigi Ricciardi: Molecular characterization of *er1* resistance to pea powdery mildew
- 40 Oksana Shtark, Sima Kumari, Reena Singh, Anton Sulima, Gulnar Akhtemova, Vladimir Zhukov, Andrey Shcherbakov, Elena Shcherbakova, Alok Adholeya, Alexey Borisov: Advances and prospects for development of multi-component microbial inoculant for legumes

## SPORT

- 45 Fédération Internationale de Fabaceaeuse Football Association (FIFFA): Second International Legume Football Cup

## ART

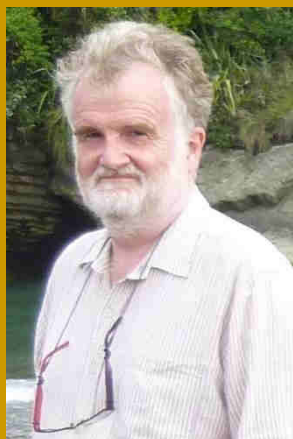
- 47 Aleksandar Mikić: Legumes of the world of Narnia

## EVENTS

- 48 Second International Legume Society Conference, Tróia, Portugal, 12-14 October 2016
- 54 Global Year of Pulses - 2016



Carte blanche  
to...



...Noel Ellis  
and



Julie Hofer


## Sgt. Legume Science Joint Club Band

**T**he International Legume Society (ILS) was formed in 2011 and had its inaugural meeting in Novi Sad in May 2013. It is concerned with all aspects of legumes; this very broad agenda grew from its origin in AEP (Association Européenne des Proteagineux) that was concerned mainly with pulses. This focus remains part of the ILS and is particularly relevant for the current issue of Legume Perspectives which occurs on the 150th anniversary of the two presentations of Mendel's work to the Brno Natural Science Society in 1865 (the February and March meetings). Next year is the 150th anniversary of the publication of his paper *Versuche über Pflanzen-Hybriden* and it is also the International Year of Pulses, so we encourage all in the ILS to use this opportunity for the promotion of legumes in diet and agriculture while also re-emphasising the importance of good genetics.

... 'Twas one fifty years ago today<sup>1</sup>  
Gregor Mendel taught our band to play  
He's been going in and out of style<sup>2</sup>  
But is guaranteed to raise a smile  
So may I introduce to you  
The act you've known for all these years  
Sgt. Legume Science Joint Club Band

We're Sgt. Legume Science Joint Club Band  
We hope you will enjoy the show  
We're Sgt. Legume Science Joint Club Band  
Sit back and let the evening go  
Sgt. Legume Science, Sgt. Legume Science  
Sgt. Legume Science, Sgt. Legume Science  
Sgt. Legume Science Joint Club Band

It's wonderful to be here  
It's certainly a thrill  
You're such a lovely audience  
We'd like to take you home with us  
We'd love to take you home

I don't really want to stop the show  
But I thought that you might like to know  
That the singer's going to sing a song  
And he wants you all to sing along  
So let me introduce to you  
The one and only Greg Mendel  
And Sgt. Legume Science Joint Club Band... 

<sup>1</sup>International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India (N.Ellis@cgiar.org; J.Hofer@cgiar.org)

<sup>1</sup>8th February 1865

<sup>2</sup>Franklin A, Edwards AWF, Fairbanks DJ, Hartl DL, Seidenfeld T (2008) Ending the Mendel-Fisher Controversy. University of Pittsburgh Press, Pittsburgh

# Hitchhiking mapping in soybean: Perspectives for more targeted future breeding

by Marina TOMIČIĆ\*, Vuk ĐORĐEVIĆ, Jegor MILADINOVIĆ, Ljiljana BRBAKLIC, Sanja MIKIĆ and Dragana TRKULJA

**Abstract:** The identification of genomic regions that have retained a signature of selection during breeding of soybean (*Glycine max* (L.) Merrill) may indicate the positions of genes related to important agronomic traits or underlying adaptation to a specific environment. This can be achieved by analysing populations comprised of ancestral and elite varieties, applying multiple outlier detection tests and population-genetic principles. The hitchhiking mapping has some advantages over other approaches, as well as several limitations. The knowledge of the consequences of selection on the soybean genome in a specific breeding program could have practical importance for future breeding and yield improvement.

**Key words:** genetic hitchhiking, selection, soybean, yield

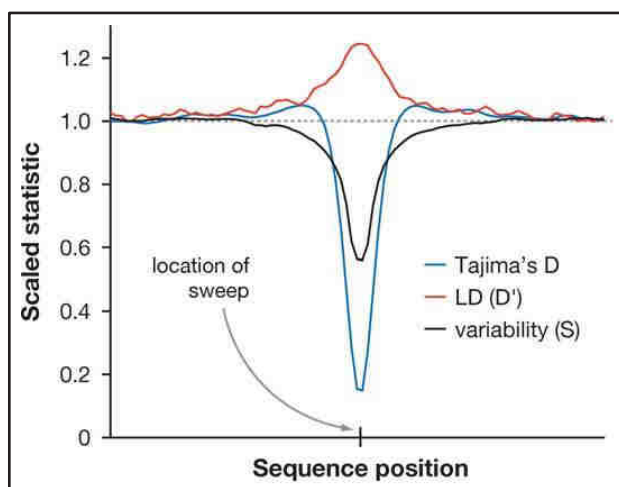
Soybean (*Glycine max* (L.) Merrill) presents highly important grain legume in the world, primarily because of the seed quality, rich in protein (40%) and oil (20%). The constant expanding of soybean acreage in the last 60 years was caused by its multiple uses, mainly as human food and animal feed, as well as source of edible oil and biofuel. In order to meet the growing needs for soybean, conventional breeding is performed through multiple cycles of selection, with the main objective to develop elite varieties, superior to their ancestors, adapted to specific growing conditions, with stable and high yield.

Long-term selection for phenotypic traits during breeding has indirectly resulted in insignificant loss of genetic diversity of agronomically important genes (10). The process by which a positive selection eliminates or reduces variation of the selectively important loci in the population is known as selective sweep (4). Except reduced variability in target genes, signatures of selection might include shift in allele frequency spectrum, increased linkage disequilibrium and increased levels of population differentiation (Fig. 1) (7). Due to the genetic linkage and consequently low recombination rates, selectively neutral loci that are linked to the targets of selection are also affected by a selective sweep and this phenomenon is marked as genetic hitchhiking (5). A population-genomic approach which utilizes a genome scan of neutral molecular markers to detect regions with signatures of selection forms the basis of the hitchhiking mapping (2, 8). The highest number of tests for the identification of loci favoured during selection compares levels of differentiation and levels of diversity between populations. The power of differentiation based tests does not decline over time as long as different alleles predominate in compared populations. On the contrary, the power of variability based tests is the highest immediately following selection, when the number of alleles is drastically reduced, and declines over time as population increases diversity. Methods for detecting selection can also be divided into model-based, that rely on assumptions about population structure and model-free, where candidate loci are identified as outliers in an empirical distribution of a summary statistic (9).

The application of the hitchhiking mapping started at the beginning of this century, aiming to determinate the genetic basis of local adaptation of natural populations. Soon after, a number of studies

have been performed to identify genomic regions responsible for the crop domestication and improvement, by comparing wild relatives and modern varieties (1). This approach has been successfully conducted in a number of major crops including wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), sunflower (*Helianthus annuus* L.), rice (*Oryza sativa* L.) and sorghum (*Sorghum bicolor* (L.) Moench). For soybean, there have been some recent studies on the effects of artificial selection, during domestication and improvement. A resequencing study of soybean indicated that 1,52% of the whole genome and 4-5% of the annotated genes were associated with significant signs of artificial selection by the modern breeding (3), confirming the hypothesis that selection affected a limited number of loci, influencing small reduction in diversity during modern soybean breeding. If a similar genome scan would be applied in breeding programmes to compare ancestral and elite soybean varieties, genomic regions that retained signatures of selection during soybean improvement in specific environments could be detected. Since the pedigrees of modern soybean varieties mainly include elite parents, it can be speculated that high and stable yield, is the only constant criteria during breeding. Considering that the plants with higher yield are better adapted to certain environments, the alleles and haplotypes that confer high yield over different growing conditions were favoured during the breeding. It is of great interest for soybean breeders to identify genomic regions that exhibit signatures of selection through the development of elite varieties, as they may represent important regions for agronomic improvement or crop adaptation, and may have implications in more targeted subsequent breeding (6).

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**Figure 1. The effect of a selective sweep on genetic variability, linkage disequilibrium (LD) and the frequency spectrum (Tajima's D) in the surrounding region (7)**

The advantage of hitchhiking mapping within breeding programmes is that it does not require creating neither mapping populations nor collecting replicated phenotypic data. By using molecular markers alone, it can take advantage of yield progress that occurred during the entire period of crop breeding, enabling the isolation of loci of more practical importance for developing varieties in target environment. In addition, there are also several challenges for successful application of this approach. Since the demographic events, such as genetic drift and population bottleneck, cause the reduction of genetic diversity, it can be difficult to separate the signatures of selection from those resulting from demographic history of a population. Further testing is necessary to be conducted, having in mind that selection acts in a locus-specific manner and demographic events have genome wide effects (10). Besides that, considering that hitchhiking mapping faces the problem of identifying false positive results, it is important to apply multiple tests that assume different concepts and hypothesis to raise the candidate status of the identified loci (9). Moreover, different approaches could identify additional regions that may be overlooked by a particular test. However, the strongest validation of positive selection will with no doubt come from functional characterization. Integration of population genomics with available genomic resources would provide information on

potential functions of genes under selection. The identified QTLs will likely be in accordance with the criteria used during selection, playing a role in the process of soybean improvement. If these loci are identified in different environments, they probably influence general productivity and could be regarded as universal. Loci identified in single environment could be considered as environment-specific, being influenced by different selective pressures within local conditions and, as a consequence, strong genotype by environment interactions.

In soybean, yield and most agronomic traits, including resistance to abiotic and biotic stress, protein concentration and oil content, are quantitative traits controlled by multiple genes, with low heritability and strong genotype by environment interaction. This greatly affects the identification of genetically superior plants based on phenotype and can be considered as one of the major hindrance during conventional breeding. The specific alleles of the identified selectively important loci may serve in creating favourable haplotypes for a target environment and possibly be used in marker-assisted breeding. Thus, the detection of selectively important genes, as well as the identification of associated molecular markers, presents the prerequisite for successful marker assisted selection that would greatly improve efficiency of breeding.

#### Acknowledgements

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# Breeding a red clover variety for grazing use

by Beat BOLLER, Franz SCHUBIGER and Peter TANNER

**Abstract:** Cultivated red clover (*Trifolium pratense* L.) is almost exclusively used for mowing. It grows too tall to fit as a companion to typical pasture grasses, and it does not tolerate intensive grazing. In 1991, we initiated a breeding programme to develop a red clover variety for grazing use. In 2010/2011, we were able to register the cultivar Pastor in Switzerland/France. Pastor originates from pasture ecotypes and breeding material of the cultivated, persistent “Mattenklee” type of red clover. Pastor is distinguished by a prostrate growth habit, strong tillering and improved grazing tolerance when grown in mixtures with grasses.

**Key words:** breeding, grazing, red clover, *Trifolium pratense*

## Introduction

Cultivated red clover (*Trifolium pratense* L.) is a high yielding forage legume mainly used for producing conserved forage in association with Italian ryegrass. However, it grows too tall to fit as a companion to typical pasture grasses, such as perennial ryegrass or tall fescue, and it does not tolerate intensive grazing. For this reason, white clover (*Trifolium repens* L.) is the usual species of choice as a pasture legume. However, white clover has some disadvantages. It tends to have unbalanced high protein content, and due to its capability of spreading through stolons, it often dominates pastures to an undesirable degree when little nitrogenous fertilizers are applied, such as in organic agriculture. Red clover is more balanced concerning protein/carbohydrates ratio in the forage and does not spread through stolons. These considerations led us to start developing a red clover variety for grazing use.

## Breeding procedure

Breeding was initiated by Agroscope at Zürich-Reckenholz in 1991 with the aim of creating a red clover variety with improved tolerance to grazing, as seen in spontaneous red clover in permanent pastures, while maintaining as much as possible of the high yield potential of cultivated red clover (1). Breeding goals were persistence under grazing for at least 3 years, a rapid regeneration after grazing through strong tillering, compatibility with pasture grasses, and an acceptable yield potential for admittance to national variety lists. An ecotype population of red clover originating from a heavily grazed pasture in the Swiss Jura region was intercrossed with breeding material of the cultivated, persistent “Mattenklee” type, followed by a backcross with Mattenklee and 3 generations of individual plant selection for prostrate growth and strong tillering. The different steps of the breeding scheme are illustrated in Table 1.

## Performance under mowing

Performance of red clover ‘Pastor’ under mowing was tested officially in the Swiss variety trials 2005-2007 (2) and 2011-2013 (4). In both series, dry matter yield, vigour, and especially competitive ability of ‘Pastor’ were rated poorer than the average of recommended varieties. However, persistence over three years of ‘Pastor’ was rated just as well as the average of recommended varieties. In the official French variety trials (3), dry matter yield of ‘Pastor’ was recorded 2% lower than diploid standards, but persistence was rated better than average and almost at the level of the best listed variety. In our own unofficial breeder’s trials, yield of ‘Pastor’ under mowing was 5% to 10% lower than the average of recommended varieties. Overall, performance of ‘Pastor’ under mowing would be insufficient to support registration or recommendation.

**Table 1. Breeding steps in the development of grazing-type red clover ‘Pastor’**

Years	Breeding step
1991-1992	Observation of individual plants of an ecotype population from a heavily grazed pasture in Undervelier, Canton Jura (“Jura” population)
1992-1993	20 pair crosses between one of 10 selected plants “Jura” and one of 20 selected plants from a population of the persistent cultivated „Mattenklee“ (Mk) type
1994-1995	Individual F <sub>1</sub> plants (Jura × Mk) in breeding nursery, 26 plants selected
1995	Pair crosses 26 plants F <sub>1</sub> (Jura × Mk) × 26 plants from a second Mk population
1996-2002	Individual F <sub>1</sub> to F <sub>3</sub> plants of the backcross [(Jura × Mk) × Mk] in breeding nursery. Selection for prostrate growth habit, small leaves, strong tillering, nematode resistance, persistence, seed yield. In each generation, insect-mediated open or pairwise hand pollination among 44 (F <sub>1</sub> ), 20 (F <sub>2</sub> ) and 42 (F <sub>3</sub> ) selected plants
2003-2004	Family selection in F <sub>4</sub> rows, 21 progenies selected
2004-2010	Performance testing under cutting and grazing
2005-2007	VAT testing for Swiss recommended list
2008-2010	VAT testing for French variety list including a special test under grazing
2007-2010	DUS testing Bundessortenamt Germany
2010	Admission in Switzerland, variety ‘Pastor’
2011	Admission in France, variety ‘Pastor’

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## Performance under grazing

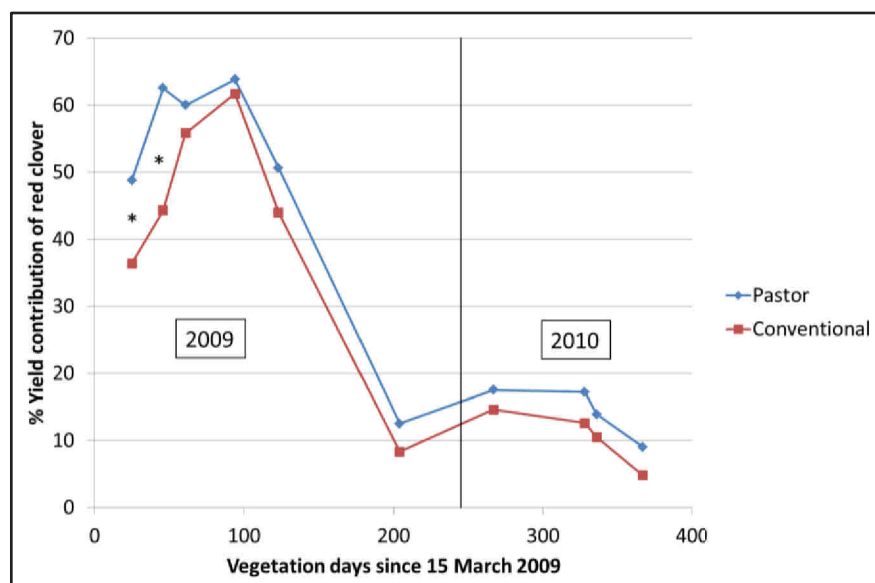
Grazing performance of 'Pastor' was compared systematically to three conventional cultivated red clover cultivars: 'Milvus', 'Lemmon' and 'Mistral' in a field trial established at Zürich-Reckenholz in August 2008. Red clover was sown at a rate of 3 kg ha<sup>-1</sup>, mixed with 15 kg ha<sup>-1</sup> tall fescue cv. 'Belfine'. Four randomly replicated plots of 2.8 m × 11.2 m were sown as part of a larger plot. The pasture was grazed by a herd of 8 to 14 suckler cows with their calves starting in October 2008, with 7 grazing periods of 4 days to 11 days in 2009 and 6 grazing periods of 3 days to 6 days in 2010. Yield contribution of red clover was estimated visually at 6 instances in 2009 and 4 instances in 2010. The number of red clover individual plants per unit surface was counted at the end of each growing season, 2008, 2009 and 2010. The number of tillers per individual plant was counted on a random sample of 8 and 5 plants per replication in autumn of 2009 and 2010, respectively. This trial was carried out as a complement to the official French variety trials (3).

At each of the 10 observations under grazing, yield proportion of 'Pastor' was higher than that of the conventional red clover cultivars (Fig. 1). The difference was highly significant in spring of 2009, and also significant overall when compared to each of the three conventional cultivars, with an average of 39.7% for 'Pastor', 35.2% for 'Milvus', 35.3% for 'Lemmon' and 28.4% for 'Mistral'. The same was true, on average over two observations, for the number of tillers per plant and especially the number of tillers per unit surface; detailed results, see (1). However, from the larger difference in tiller number compared to differences in yield proportion, it can be inferred that each tiller contributed less yield for 'Pastor' than for the conventional cultivars. This was due to the smaller leaves and shorter petioles of 'Pastor'.

Although 'Pastor' tolerated grazing better than conventional cultivars, its yield contribution decreased markedly towards the end of the second year of stand (2009) and remained at a rather low level in the third year of stand (2010). Boller et al. (1) observed a greater presence of flower heads with ripening seeds after the September grazing period with 'Pastor' than with conventional red clover. Allowing self-reseeding in such a situation might help providing greater persistence.

## Conclusions

For the first time in Europe, a red clover bred specifically for grazing use was admitted to official and recommended lists: 'Pastor'. It presents a compromise between the high yield potential of the Swiss "Mattenklee" type of red clover and the adaptation to grazing of ecotypes from heavily grazed permanent pastures. The yield potential of 'Pastor', as measured under mowing, is only slightly lower than that of conventional red clover cultivars. It persists under grazing for at least three years, maintaining a higher proportion in a mixed grass-clover sward than conventional cultivars. If it is desired that a pasture with 'Pastor' lasts longer than 3 years, it will be necessary to allow for self-reseeding.



**Figure 1. Yield contribution of red clover, estimated visually in a mixed red clover-tall fescue sward grazed by suckler cows in the second (2009) and third (2010) year of stand, for grazing-type red clover 'Pastor' compared to the average of three conventional red clover cultivars ('Milvus', 'Lemmon' and 'Mistral'); vertical line: end of vegetation on 15 November 2009, beginning of vegetation on 15 March 2010**

\*Difference significant ( $p < 0.001$ )

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# Hay quality of Persian clover (*Trifolium resupinatum* var. *resupinatum*) affected by soil properties

by Ozlem ONAL ASCI<sup>1</sup>, Fatih NALBANTO<sup>1</sup>, Metin DEVECİ<sup>1</sup> and Zeki ACAR<sup>2</sup>

**Abstract:** The plant samples of Persian clover (*Trifolium resupinatum* L. var. *resupinatum* Gib & Belli.), growing naturally in the coastal districts of Fatsa, Gulyalı, Merkez, Persembe and Unye of the Ordu Province in Turkey, were picked up from rangelands at flowering stages in 2010 and 2011. Dry weight of per plant and crude protein, acid detergent fibre, neutral detergent fibre, ash, Ca, Mg, K and P content in hay, Ca / P and K / (Ca + Mg) and relative feed value were determined. Some soil properties of rangelands were also determined. The results showed that *Trifolium resupinatum* L. var. *resupinatum* plants provide high quality hay for ruminants when harvested or grazed in flowering. Moreover, there was a strong relationship between soil properties and hay quality.

**Key words:** ADF, mineral contents, NDF, Persian clover

## Introduction

Persian clover (*Trifolium resupinatum* L. var. *resupinatum* Gib & Belli.) is an annual winter plant, but it can be biennial when environmental conditions are appropriate. It has great regrowth feature after grazing and cutting and thus has high feeding value as a pasture or hay (4). It is present in rangelands but is not commonly cultivated in Turkey. By this reason, in the scope of this study yield, nutrient content and some quality features of Persian clover growing naturally in coastal meadows in Ordu Province were determined and the correlation between soil properties and plant nutrients was investigated.

## Material and methods

Persian clover plants, growing naturally in the coastal districts of Fatsa, Gulyalı, Merkez, Persembe and Unye of the Ordu Province in Turkey, were picked up in 2010 and 2011. In the sample areas, the soil texture was changing from silty clay to sandy clay loam. While the soil organic matter content changed from little to high (1.18 % - 3.61 %), the phosphorus content varied from 0.8 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> to 195.8 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>. The potassium content was high (346 kg ha<sup>-1</sup> -1620 kg ha<sup>-1</sup> K<sub>2</sub>O) all rangelands. The pH values varied from 5.8 to 7.65.

The long term (1970-2011) annual rainfall and mean temperature of Ordu province were 1042.7 mm and 14.3 °C, respectively.

Randomly, at least 15 plants were collected from each district in flowering each year. The plant samples were dried at 60 °C and ground to pass through 1 mm screen.

The crude ash content was determined by ashing at 550 °C for 4 h (11). The dry matter (DM), crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), Ca, P, Mg and P content of samples were determined by using Near Reflectance Spectroscopy (NIRS, 'Foss 6500') with software package program 'IC-0904FE'. The Relative Feed Value (RFV) of the samples was calculated as follows:

$$\text{DMI (\%)} = 120 / \text{NDF}$$

$$\text{DDM (\%)} = 88.9 - 0.779 \times \text{ADF}$$

$$\text{RFV (\%)} = \text{DDM} \times \text{DMI} \times 0.775 \text{ (6)}$$

The quality standards of legume hays are given in Table 1 (10). All the data are presented as a mean and standard deviation determined by using SPSS 10.0 Statistical Package Program. The results are given as a mean of two years. Both chemical and quality results are reported on a dry matter basis.

**Table 1. Quality standards of legumes, grasses and legume-grass mixtures (10)**

Quality standard	CP (% DM)	ADF (% of DM)	NDF (% of DM)	RFV
Prime	> 19	< 31	< 40	> 151
1 (Premium)	17-19	31-35	40-46	151-125
2 (Good)	14-16	36-40	47-53	124-103
3 (Fair)	11-13	41-42	54-60	102-87
4 (Poor)	8-10	43-45	61-65	86-75
5 (Reject)	< 8	> 45	> 65	< 75

CP: crude protein, ADF: acid detergent fibre, NDF: neutral detergent fibre, DM: dry matter, RFV: relative feed value

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Additionally, a correlation analysis was used to determine the relationship between plant and soil properties.

## Results and discussion

The values of yield, chemical composition and relative feed value (RFV) of Persian clover plants are given in Table 2. The dry weight (DW) varied between 2.39 g plant<sup>-1</sup> and 6.41 g plant<sup>-1</sup>. The lowest DW was in Unye, while the highest was in Gulyalı (Table 2). This could be due to different ecological conditions, genetic variance and capability.


The crude protein, ADF and NDF content in hay are important properties to assess hay quality (3). The crude protein content in Persian clover plants varied from 20.01% to 23.18% (Table 2). A large variation of the ADF and NDF content was also determined (Table 2). While the minimum ADF content (33.67%) was obtained from plants collected from Merkez, the minimum NDF value (43.53%) was determined in those from the Gulyalı district. The RFV of Persian clover hay changed between 113.6 and 133.7. The CP, ADF and NDF contents and RFV of Persian clover hay indicated that it may produce high quality forage (10).

Minerals are also important for animal health. The average crude ash, Ca, Mg, K, and P contents and Ca / P and K / (Ca + Mg) ratios were 11.47, 1.85, 0.44, 2.52, 0.42, 4.42 and 1.10, respectively (Table 2). It is reported that the requirements for gestating or lactating beef are 0.18% - 0.44% for Ca, 0.04% - 0.1 % for Mg, 0.6% - 0.8% for K and 0.18 % - 0.39% for P (9, 12). While determined ratios of Ca, Mg and K were very high, that of P was similar compared to recommended ratios in hay. Although the generally recommended Ca / P is 2:1, it ranges in dietary 1:1 and 7:1, resulting in

similar performance in ruminants (2), if adequate vitamin D is available (1). Similarly, the K / (Ca + Mg) value in forage of 2.2 or higher can cause tetany (7). Our results indicated that while Ca : P milk fever is not seen in livestock when adequate vitamin D supplied, the hay K / (Ca + Mg) ratio was under the level of tetany.

As expected, the strongest relationship is determined (0.98) between RFV and NDF according to correlation analysis. It is followed by the relationship of RFV and ADF (0.96) (Table 3). Adversely inverse relationship is found between soil pH and P content of the plant. Soil pH affects plant nutrient uptake. If the soil pH exceeds 6, the amounts of P uptake decrease. P exists in the structure of guanosine-triphosphate (GTP) required for cellulose synthesis (8). Phosphorus is an essential ingredient for *Rhizobium* bacteria to convert atmospheric N into an ammonium form useable by plants and, thus, increases yield and nitrogen content in legumes. By this reason, the relationship between P content of the soil and ADF, CP, K, P and ash in plant is very important. Additionally, a reverse way relationship between K content of the soil and Ca, Mg, P, CP, ash content of the plant is determined. It is well documented that a plant takes up K beyond its needs if high levels of available K is presented in soil. If K is found high amount, plants take up less Ca and Mg (5).

## Conclusion

The CP, ADF and NDF contents and RFV of hay indicate a high quality forage. Also the results of study showed that although the soil had much K, K / (Ca + Mg) ratio of hay was under level of tetany and only milking cattle should be grazed to avoid to milk fever where *Trifolium resupinatum* L. var. *resupinatum* is dominantly existed in vegetation. 

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**Table 2. Dry matter yield and some quality components in Persian clover**

Districts	DW (g plant <sup>-1</sup> )	CP (%)	ADF (%)	NDF (%)	RFV (%)	Ash (%)	Ca (%)	Mg (%)	K (%)	P (%)	Ca / P	K / (Ca + Mg)
Gulyalı	6.41	20.01	34.55	43.53	133.67	10.16	1.80	0.40	2.41	0.40	4.50	1.11
Merkez	3.59	20.48	33.67	44.77	131.77	10.20	1.74	0.39	2.27	0.41	4.34	1.08
Persemebe	4.07	23.18	36.92	47.04	120.03	11.71	1.81	0.45	2.63	0.42	4.33	1.16
Fatsa	3.54	22.18	36.20	45.71	125.19	11.68	1.88	0.45	2.67	0.42	4.57	1.15
Unye	2.39	21.96	38.29	48.79	113.60	11.86	1.83	0.43	2.57	0.42	4.44	1.15
Mean	3.97	22.43	36.34	46.17	123.51	11.47	1.85	0.44	2.52	0.42	4.42	1.10
SD	5.75	3.80	3.76	4.06	16.86	2.40	0.16	0.09	0.40	0.04	0.59	0.19

$n = 208$  for DW,  $n = 87$  for quality parameter

**Table 3. The simple correlation coefficients ( $r$ ) among plant and soil properties in Persian clover**

		Plant Properties										Soil Properties			
		Ca	K	Mg	NDF	P	CP	RFV	Ca/P	K / (Ca + Mg)	Ash	pH	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	OM
Plant Prop.	ADF	0.38**	0.03	0.51**	0.92**	-0.09	0.26*	-0.96**	0.37**	-0.27*	0.32**	0.11	0.22*	-0.17	0.34**
	Ca		0.17	0.69**	0.29**	0.30**	0.65**	-0.34**	0.53**	-0.40**	0.38**	-0.1	0.20	-0.43**	0.05
	K			0.24*	-0.07	0.60**	0.63**	0.09	-0.39**	0.82**	0.40**	-0.08	0.22*	-0.06	0.02
	Mg				0.32**	0.27*	0.66**	-0.41**	0.27*	-0.28**	0.41**	0.15	0.14	-0.45**	-0.01
	NDF					-0.18	0.17	-0.98**	0.38**	-0.29**	0.27*	0.01	0.20	-0.03	0.38**
	P						0.79**	0.17	-0.64**	0.38**	0.35**	-0.27*	0.43**	-0.28**	-0.16
	CP							-0.18	-0.18	0.17	0.47**	-0.19	0.39**	-0.37**	-0.09
	RFV								-0.40**	0.35**	-0.28*	-0.04	-0.19	0.10	-0.35**
	Ca / P									-0.63**	-0.03	0.13	-0.20	-0.07	0.16
	K / (Ca + Mg)										0.12	-0.05	0.08	0.23*	0.01
	Ash											-0.21	0.23*	-0.22*	0.1
Soil Prop.	pH												-0.36**	0.15	0.23*
	P <sub>2</sub> O <sub>5</sub>													-0.05	0.30**
	K <sub>2</sub> O														0.28**

\* $p < 0.05$ , \*\* $p < 0.01$

# Use of translational genomics to identify genes important for legume seed development

by Karine GALLARDO<sup>1</sup>, Isabelle d'ERFURTH<sup>1</sup>, Christine LE SIGNOR<sup>1</sup>, Vanessa VERNOUD<sup>1</sup>, Grégoire AUBERT<sup>1</sup>, Julia BUITINK<sup>2</sup>, Jérôme GOUZY<sup>3</sup>, Jean-Marie PROSPERI<sup>4</sup>, Judith BURSTIN<sup>1</sup> and Richard D. THOMPSON<sup>1</sup>

**Abstract:** We have exploited the extensive synteny between the model legume *Medicago truncatula* and the cultivated garden pea, *Pisum sativum*, to identify loci controlling seed filling and seed composition in the crop species. QTLs for these traits are mapped by analyzing variation within collections of recombinant inbred lines. Candidate genes within the QTL intervals are identified by reference to the *M. truncatula* genomic sequence. This approach was used to uncover an endosperm subtilase that is associated with syntenic seed weight QTLs in *Medicago* and pea, and we discuss the possible role played by this enzyme in contributing to final seed weight.

**Key words:** endosperm, *Medicago truncatula*, pea, QTL, seed weight, subtilase

The model legume *Medicago truncatula* Gaertn. (Mtr) belongs to the Vicioid family of cool-season legumes that include pea (*Pisum sativum* L.), faba bean (*Vicia faba* L.) and chickpea (*Cicer arietinum* L.). Interestingly, the protein composition of seeds of pea, faba bean, and *M. truncatula* is very similar, with low levels of the essential sulfur-containing amino acids. These observations suggest that mechanisms studied in Mtr will be applicable to the crop species pea and faba bean.

In order to identify genes involved in seed yield and protein composition, we are combining QTL mapping with proteomics and transcriptomics analyses of the developing seed (7, 14). Many of the QTL obtained for these characters in Mtr and pea co-localize, confirming synteny between the two species (2, 12). Thus, seed traits are mapped in recombinant inbred line populations, and the QTL intervals are examined for seed-expressed genes. Once such gene candidates have been identified, they are studied using mutant resources such as TILLING populations in Mtr and in pea (4, 9), or TnT1 insertions for Mtr (3).

By analyzing seed weight variation in a RIL population of *Medicago truncatula* derived from the cross between Jemalong 6 and DZA315.16, a QTL for seed weight with a LOD score > 3 was identified (12). 18 seed-expressed genes were located within the QTL interval. Among these, a subtilase (MtSBT1.1) was the only gene specifically expressed in the seed at early developmental stages (5). Both MtSBT1.1 and its putative pea ortholog, PsSBT1.1, are restricted to the peripheral endosperm of the developing seed (Fig. 1). PsSBT1.1 is also located within a QTL interval for seed weight. A linkage between SBT1.1 and seed weight was also seen in association studies surveying both Mtr and pea stock centre core collections (5).

A series of TILLING mutants is available for the two genes, and we focused on 6 missense mutations for characterization, 3 for pea and 3 for Mtr. The mutations were both located in highly conserved domains of the subtilase and alteration of protein function is predicted. All Mtr and pea SBT1.1 mutant lines were backcrossed twice to WT to reduce background mutation effects, and seed weights determined. All mutant lines display reduced seed weights compared to seed from WT lines, with heterozygous lines giving intermediate values indicating dose-dependent effects of the mutant alleles. To determine the basis for the reduced seed size, the MtrP330S mutant was analyzed by light microscopy of cotyledon sections for cell number estimation, and by observation of stripped epidermal surfaces for cell area measurement. The mutant cotyledon cells are similar in size to those of wild type embryos, but significantly reduced in number (Fig. 2). In conclusion, MtSBT1.1 appears to act by determining the number of cells in the mature embryo, consistent with a role in controlling cell divisions. The final cotyledon cell number was previously identified as an important determinant of storage capacity in pea (10), and thus related to final seed weight. In contrast, there was no change in seed carbon (C) or nitrogen (N) contents, *ie.*, in overall composition of the seed, suggesting that whereas sink size was affected metabolic equilibria were not.

Subtilases play several roles in plants, including in senescence, germination, remobilisation, and programmed cell death (13). SBT1.1 may be involved in the remobilization of proteins deposited in the endosperm during its resorption to provide nutrients for the developing embryo. However, no structural difference was seen between WT and mutant Mtr endosperm at 16 DAP, suggesting that SBT1.1 does not participate in endosperm degeneration.


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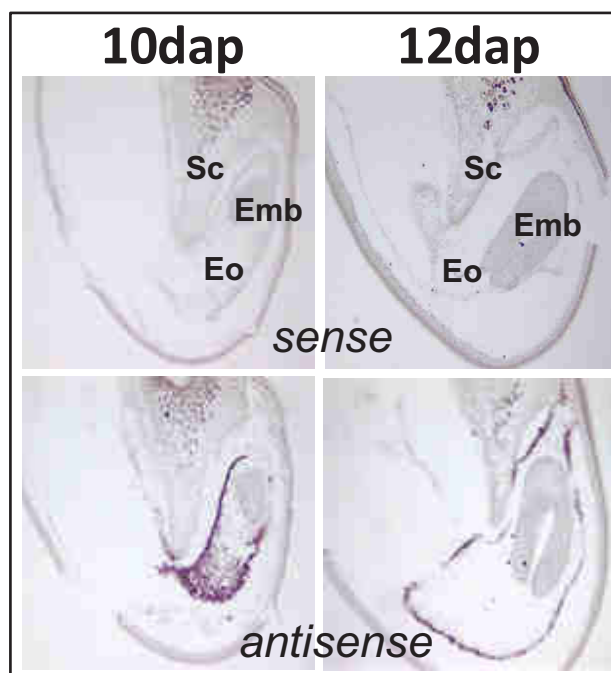
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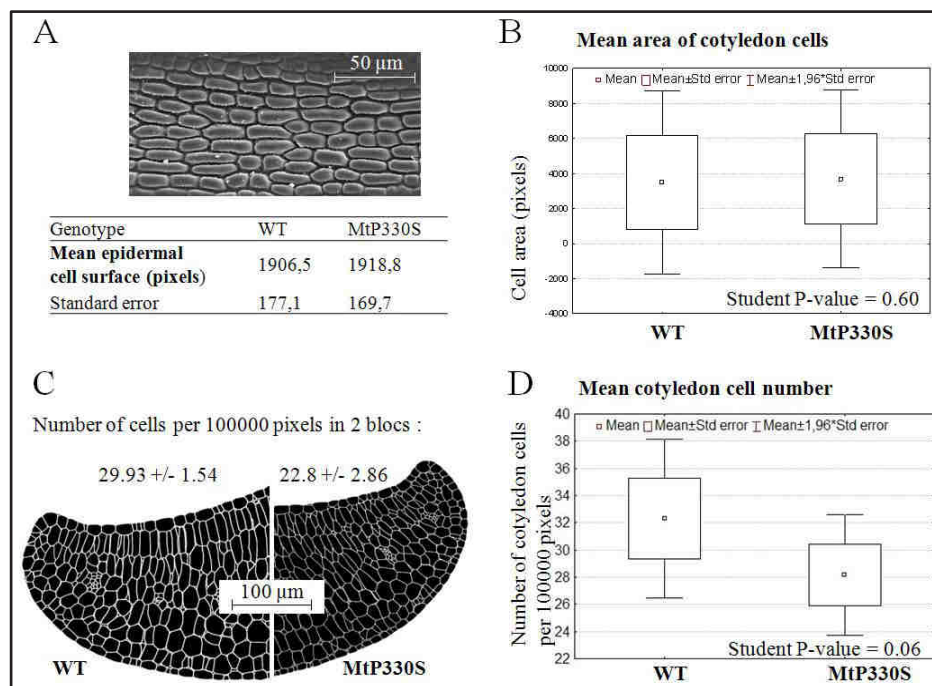
Subtilases are also implicated in the generation of specific signals, by maturation of pro-peptides. The signals may be peptides, or phytosulfokines, which stimulate cell division (8). Interestingly, in *Arabidopsis*, the AtPSK4 precursor was specifically cleaved by AtSBT1.1 (11), and a sequence related to AtPSK4 is located within a seed weight QTL interval in *Medicago* (5). The timing of expression of MtSBT1.1 and of PsSBT1.1 during the latter phase of embryogenesis, is consistent with a role in stimulation of cell division. A *CLAVATA3 / EMBRYO SURROUNDING REGION-RELATED*, *CLE8* peptide ligand, that regulates embryo and suspensor proliferation, and influences seed size, was recently identified in *Arabidopsis* and attributed a role in embryo-endosperm communication (6), suggesting a further possible substrate for SBT1.1 in legume seeds that could result in a similar phenotype to that observed. Finally, the allelic variation seen for PsSBT1.1, and its association with seed weight, may provide useful markers for selecting for this character in pea breeding populations. 



**Figure 1.** *In situ* hybridization experiments of 10 and 12 days after pollination (dap) *Medicago truncatula* seeds with *MtSBT1.1* sense (upper) and antisense (lower) probes; Emb = embryo; Eo = cellular endosperm; li = inner integuments; Mr = micropylar region of integuments; Nu = nucellus; Oi = outer integuments; reproduced from d'Erfurth et al. 2012, reference 5

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**Figure 2.** Surface area and number of cotyledon cells in mature *Medicago truncatula* seeds of the homozygous MtP330S mutant vs wild-type: (a) mean epidermal cell surface area of mature cotyledons in pixels for at least 600 cells determined from scanning electron microscopy images as shown (from 9 to 10 seeds analyzed per genotype); (b) mean comparison of cell surface area (in pixels) measured on 0.5- $\mu$ m-thick sections from cotyledon embedded tissues observed under light microscopy, data acquired using ImageJ from three biological replicates; (c) typical binary image showing the reconstruction of cotyledons from mature seeds of the homozygous MtP330S mutant and wild-type lines made by merging the different images obtained after light microscopy of cotyledons embedded in Epon resin (magnification  $\times 20$ ) and stained with toluidine blue; (d) box plot for comparison of mean cell number per cotyledon section in the MtP330S and wild-type mature seeds after reconstruction of cotyledons from mature seeds of the homozygous MtP330S mutant and wild-type lines; reproduced from d'Erfurth et al. 2012, reference 5

# The use of next-generation sequencing technologies in legumes

by Sara FONDEVILLA

**Abstract:** Next-generation sequencing (NGS) technologies are enabling fast and cost-effective sequencing of millions of reads, impressively increasing the genomic tools available for legume crop species. Among other uses, they are being used for fast and economic *de novo* sequencing of genomes or complete transcriptomes, massive identification of molecular markers, more accurate transcriptomic studies and miRNA discovery and analysis. In parallel with NGS improvement, bioinformatics, statistics and high-throughput genotyping platforms are also evolving to handle and analyse the huge amount of data yielded by these technologies. This review summarizes the implementation of NGS in legumes.

**Key words:** genomic resources, legumes, molecular markers, NGS, sequencing

## Introduction

Legumes have been called “orphan crops” due to the low investment in research in these crops compared to others such as cereals. This orphanhood has been especially dramatic in the case of the development of genomic resources. Until recently only a few sequences were available for most legumes. In the last years, the advent of Next Generation Sequencing technologies (NGS) is changing drastically this scenario, enabling the massive parallel sequencing of millions of small DNA fragments at a relative low cost. Several platforms are available such as 454, Illumina, SOLiD, Ion Torrent and PacBio. Those called “2nd generation”, requires amplification of the template molecules prior to sequencing, while the emerging “3rd generation” sequence directly individual DNA molecules. In this review the most outstanding uses of NGS on legumes will be summarized.

## De novo genomes and transcriptomes sequencing

NGS techniques are facilitating *de novo* sequencing of genomes and transcriptomes of organisms in a fast and cheap way, thus increasing enormously the genomic resources available for non-model organisms. The first legumes genomes sequenced were those of the model species *Medicago truncatula* Gaertn. and *Lotus japonicus* (Regel) K. Larsen, yielded after a long and expensive process. Nowadays, the advances in new sequencing technologies have remarkably increased the number of legume species sequenced. Thus, the genomes of chickpea (*Cicer arietinum* L.), pigeonpea (*Cajanus cajan* (L.) Huth.), soybean (*Glycine max* (L.) Merr.), common bean (*Phaseolus vulgaris* L.), and mungbean (*Vigna radiata* (L.) R. Wilczek) have been released, as the transcriptomes of chickpea, faba bean (*Vicia faba* L.), lentil (*Lens culinaris* Medik.), lupin (*Lupinus* spp.), pea (*Pisum sativum* L.), peanut (*Arachis hypogaea* L.), pigeonpea, soybean and common bean (3, 5, 7, 9). The sequencing of legumes genomes and transcriptomes is increasing our knowledge about their genome structure and their level of synteny, allowing the discovery of new genes, comparative genomics and facilitating marker assisted breeding.

## Transcriptomics

Furthermore, NGS are being used to achieve unprecedented levels of accuracy in transcriptomic studies. RNA-seq technique, that involves the sequencing of all transcripts expressed in a given situation, is not only a powerful tool for *de novo* transcriptomes sequencing, but also to identify genes differentially expressed in two different situations. As examples, this technique was used to identify genes differentially expressed in seed development in *M. truncatula*, nodulation in soybean, drought in common bean and flower development in chickpea

(8). In addition, NGS technologies can be combined with reduced complexity transcriptome techniques such as SSH, cDNA-AFLP, SuperSAGE or MACE for and increase coverage of genes obtained by these techniques. This approach has been used, for example, to decipher the genes involved in drought tolerance, ascochyta blight resistance or symbiosis with rhizobium in legumes (4).

## Molecular markers

First legume linkage maps were formed by a limited number of markers. The first markers included were morphological markers and isozymes, then followed by anonymous DNA markers or low reproducible markers, such as RAPDs (Random Amplified Polymorphic DNA) and RFLPs (Restriction Fragment Length polymorphism). The development of first SSRs (Simple Sequence Repeats), highly polymorphic and reproducible, was a great advance but still their identification was highly time-consuming. NGS are enabling the identification of molecular markers in a high throughput way, thus increasing tremendously the number of molecular markers available in legumes. The sequencing and re-sequencing of several accessions made possible the massive identification of SSR and single nucleotide polymorphism (SNP). In addition, NGS combined with restriction enzymes to reduce genome complexity, as done in RAD (Restriction Site Associated DNA)-seq technique, can also be used for high-density SNP discovery and genotyping in large populations. Hundred of loci containing repetitive elements have been identified in pea, faba bean, chickpea, common bean, groundnut and pigeonpea. Millions of SNPs have been identified in *M. truncatula* and thousands of them in chickpea, soybean, pea, common bean and pigeonpea (10). NGS derived markers are being applied in the development of high saturated maps, accurate genetic diversity studies and genome wide association mapping.

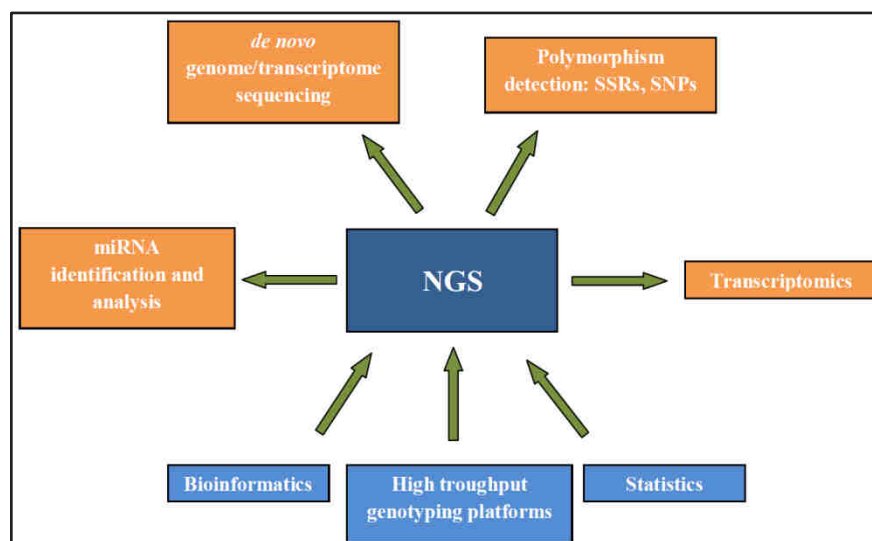
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## miRNA analysis

The unprecedented level of sequencing depth obtained by NGS is discovering new RNA species. By sequencing the small fraction of RNA, new small RNAs species are emerging. Their analysis is showing that small RNAs (sRNAs), such as microRNAs (miRNAs), small interfering RNAs (siRNAs), and Piwi-interacting RNAs (piRNAs), play an important regulatory role in many biological processes. miRNAs are small 21 nt - 26 nt species of non-coding RNAs that regulate gene expression post-transcriptionally by controlling mRNA translation or stability. Through complementary binding to specific target genes they play important roles in plant development and response to biotic and abiotic stresses. The deep sequencing obtained by NGS, combined with the availability of genome and transcriptome sequence data, and the development of improved bioinformatic tools for miRNA prediction, is allowing the identification of conserved and low-abundance species-specific miRNA, quantifying its level of expression and prediction of their targets. miRNA have been identified in *M. truncatula*, soybean, common bean, peanut, *Vigna mungo* and chickpea, in most cases using NGS approach (6). They have been shown to be involved in symbiosis, response to nematodes and aluminum stress (1).

## Associated fields

NGS are yielding a huge amount of data. The challenge now is how to make sense of this information. In parallel with the evolution of NGS technologies other disciplines are evolving to meet this demand. Bioinformatics is a need for handling these huge amount of data, more accurate software are being created for quality control, read mapping, assemble and quantification of the yielded million of sequences; new statistical algorithms are also continuously being developed to respond to the specific characteristic and distributions of NGS created data. High throughput sequencing identifies and unseen number of polymorphism but new, also high throughput and cost-effective technologies, are needed to genotype these polymorphisms in wide populations, required to develop genetic maps or perform diversity or genome wide association studies. Several technologies and platform are available for SNPs high-throughput genotyping such as GoldenGate, Infinium and KASPar assays, Affymetrix SNP array, High Resolution Melting Curve or Genotyping by Sequencing, among others. GoldenGate assays are available



**Figure 1. Main uses of next-generation sequencing technologies (NGS) in legumes**

for pea, chickpea, common bean, cowpea and lentil as well as a KASPar assays for faba bean (2).

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# Transcriptional profiling comparison of related grain legume genes differentially regulated in response to infection with *Ascochyta* spp.

by Nuno F. ALMEIDA<sup>1\*</sup>, Diego RUBIALES<sup>2</sup> and M. Carlota VAZ PATTO<sup>1</sup>

**Abstract:** Legumes are high quality protein sources and important ecological services providers. However their yields are affected by several pathogens, being ascochyta blight one of the most devastating foliar diseases. The deepSuperSAGE has been employed to increase transcriptomic resources for supporting resistance breeding. By comparing grass pea (*Lathyrus sativus* L.) to pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.) deepSuperSAGE transcriptome response to ascochyta blights, the same up-regulation of the ethylene pathway and chitinase transcripts was detected in grass pea and pea. No common responses were spotted between grass pea and faba bean. This first genome-wide comparison of gene expression yielded a valuable set of candidate genes for future legume resistance breeding.

**Key words:** ascochyta blight, deepSuperSAGE, faba bean, grass pea, pea

All over Europe there is a growing concern among consumers and policy-makers about the safety and impact of agriculture on the environment. Grain legumes can contribute to an environmentally friendly agriculture due to naturally fixing atmospheric nitrogen, providing this vital nutrient for their growth and maintaining soil fertility for subsequent crops in rotations. Also, as break-crops in rotation, grain legumes slow down the build-up of pests, diseases and weeds and reduce the need for pesticides in subsequent crops.

In addition, when resistant legume genotypes exist, the reduction on pesticides is even bigger, due to the plant endogenous capability to restrain pathogen growth. As results of the pathogen's inability to complete its life cycle, the pathogen total population will be reduced. However, the absence of sources of complete resistance against devastating foliar diseases, such as ascochyta blight is a real problem in some important legume crops as e.g. pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.). In this case, the elucidation of the common resistance responses among related legume species would help to identify candidate resistance genes for future legume precision breeding.

Much of our team work has been focused on grass pea (*Lathyrus sativus* L.) / pathogen interactions. Grass pea is a multipurpose robust grain legume crop phylogenetically related to pea (8) that despite its underuse have high nutritional value and a great potential for harsh environments. It is also recognized as potential source of resistance to several important legume pathogens (11), such as ascochyta blight, powdery mildew, rust and bacterial blight (12). As a result, this robust crop is rightly considered as a model for sustainable agriculture (12), but with just a few molecular breeding tools developed specifically for this species.

Within the framework of the ERA-NET Plant Genomics project LEGRESIST, deepSuperSAGE was applied to elucidate three different legume-ascochyta blight interactions, *Lathyrus sativus* - *Ascochyta lathyræ* Trail (1), *Vicia faba* - *Ascochyta fabae* Speg. (5) and *Pisum sativum* - *Didymella pinodes* (Berk. & A. Bloxam) Petr. (4).

Ascochyta blights are among the most important pea and faba bean diseases worldwide (7). In faba bean, *A. fabae* has a global distribution causing up to 90% yield losses in susceptible cultivars. In pea, ascochyta blight is caused by a complex of fungi formed by *D. pinodes*, *Ascochyta pisi* Lib., *Phoma medicaginis* var. *pinodella* (L.K. Jones) Boerema and *Phoma koolunga* J.A. Davidson, D. Hartley, Priest, S. Kaczyn., Herdina, A. McKay & E.S. Scott, with *D. pinodes* the most predominant and damaging of them. On the other hand, grass pea quantitative resistance to ascochyta blight caused by *D. pinodes*, seems to be controlled by two independently segregating genes, operating in a complementary epistatic manner (9).


The deepSuperSAGE (6) is the combination of SuperSAGE with high-throughput sequencing technologies, allowing genome-wide and quantitative gene expression profiling. In this sense it is a digital gene expression profiling method, that generates for each cDNA molecule, one 26 bp-tag through the type III restriction enzyme *Eco*P15I. These tag sequences are characteristic for the cDNA they were derived from and can be assignment to specific genes. Due to its high discrimination power, this SuperSAGE technology is especially useful in host-pathogen interactions expression studies since tags can be obtained from infected tissue from host and pathogen during their interaction and unambiguously assigned to either of them, giving insight into the time frame of the cross talk between the organisms under natural conditions.

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When analysing the deepSuperSAGE results obtained with the different grain legume species under the frame of the LEGRESIST project, unfortunately it was not possible to directly compare the identified resistance genes between faba bean and grass pea or pea response to ascochyta blight, due to the fact that different approaches were employed in data analysis. While Madrid et al. (5) categorized the faba bean genes by gene ontology (GO) terms, the grass pea and pea deepSuperSAGE transcriptomes were categorized using the software Mapman (10). GO terms are used to analyse gene functions and attribute then a functional category, while Mapman allows the categorization of the functionally annotated genes into several pathways and their physiological roles and networks.

Nevertheless since the same approach was used for grass pea and pea data analysis it was possible to perform a deeper comparison between these two plant-pathogen transcriptomics analysis. As a result of this detailed assessment it was observed in both cases, and upon ascochyta blight infection, an induction of the ethylene (ET) pathway and up-regulation of the enzyme chitinase (1, 4). Thus, up-regulation of ET signaling and overexpression of chitinases may be a general response of these temperate legumes to ascochyta blight infection. Induction of the ET pathway is described as a common defence response against necrotrophs (2) such as *Ascochyta* sp., while the production of chitinases are among the vast array of antimicrobial compounds that limits colonization by the pathogen (3).

These studies provided a high throughput overview of the gene expression profile of the grass pea, pea and faba bean response to ascochyta blight and allowed, to a certain extent, to recognize common resistance responses among related grain legume species. The transcription profiling of candidate genes identified via deepSuperSAGE in larger sets of resistant and susceptible germplasm will deepen our knowledge on those genes conferring resistance to ascochyta blight. In addition, the genomic resources here developed can be combined with the continuous increasing genomic data to deliver functional molecular markers to be used in linkage map development, QTL analysis and association mapping of other economically important traits in those crops. 

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# Soybean quality: Adaptation to European needs

by Johann VOLLMA<sup>1\*</sup>, Daisuke WATANABE<sup>1</sup>, Martin PACHNER<sup>1</sup>, Jonibek KHUDAYKULOV<sup>2</sup> and Tomáš LOŠÁK<sup>3</sup>

**Abstract:** On the world-wide scale, soybean (*Glycine max* (L.) Merr.) is considered as an oilseed crop. In contrast, the primary interest of European soybean production is in its high protein content for providing a versatile raw material for livestock feed, food industry and traditional soy-food production. Thus, while oil compositional traits play a major role in North American soybean research, other seed traits appear to be relevant in Europe. Soybean genetic variation in seed compositional traits can therefore be utilized in plant breeding programs for fine-tuning seed characteristics to the specific needs of the industries and utilization patterns involved.

**Key words:** food-grade soybean, genetic diversity, seed protein content, soybean breeding

## A glimpse of history of soy quality

Since the mid 19th century, soybean (*Glycine max* (L.) Merr.) had been termed the “Chinese oil pea” (*pois oleagineux*) particularly in France which takes into account its significant oil content unusual to most other legume crops. In the aftermath of the 1873 World Exhibition in Vienna, Austria, Friedrich Haberlandt did not only recognize the agronomic potential of soybean in a series of experiments across Central Europe,

but he also reported on the high seed protein content of up to 40% and proposed a number of processing options based on the remarkable composition of the soybean seed. In 1880, however, Edmund von Blaskovics of Mosonmagyaróvár, Hungary, described a disappointing weight gain when feeding cattle with raw soybeans. Despite the high nutritional value of soybean, the limited feeding performance was due to antinutritional factors which were only overcome more than three decades later when Osborne and Mendel found out that steam cooking of soybeans greatly improved the digestibility of soy meals.

## Soybean quality for feed and food

On average, the soybean seed contains about 40% protein and 20% oil. In contrast to other legumes, soybean is practically free of starch whereas it contains significant levels of soluble carbohydrates such as sucrose and glucose as well as the oligosaccharides raffinose and stachyose (3). In North America, soybean oil quality is of major interest: While linoleic and oleic acids are the predominant fatty acids in soybean oil, genotypes have been developed with reduced linolenic acid (C18:3) to avoid trans-fatty acid formation during oil processing (1). Other strains have been selected from mutant populations with either reduced or enhanced concentration of saturated fatty acids in order to better adapt soybean oil to particular health and processing needs.

In Europe, soybean is mainly grown for its high seed protein content, while vegetable oils are produced from oilseed rape, sunflower, olive and a number of regional specialty oil crops. Both genetic and environmental variation is high in soybean seed protein content when growing early maturity soybeans (maturity groups 000 to I) in Central Europe: Protein contents from below 30% to 48% have been reported (8).

Low protein content might be due to unfavorable environmental conditions and reduced rates of biological di-nitrogen fixation. It has been estimated that only 40 to 52% of total nitrogen uptake of soybean is from symbiotic di-nitrogen fixation, while the remainder is from soil nitrate uptake (6). The comparatively low di-nitrogen fixation rate which has implications on seed quality and on the crop rotation value of soybean as compared to other legumes is well in agreement with a meta-analysis on soybean nitrogen fixation which revealed a negative nitrogen balance in 80% of cases across a high number of individual studies (4).

Over 90% of soybean is utilized in animal feeding rather than in human food production at present. For developing a soybean for on-farm utilization for livestock feeding, reductions in oil content and trypsin inhibitor activity are necessary. Germplasm carrying a null-allele at the Kunitz trypsin inhibitor locus has been utilized to develop a Kunitz trypsin inhibitor free soybean with a clearly reduced total trypsin inhibitor activity (9). However, genetic variation in a second inhibitor protein, the Bowman-Birk protease inhibitor has not been found so far which is limiting the progress towards developing a soybean for livestock feeding without the need for feed pre-processing. In addition, an oil content of 17-20% appears too high for full-fat soybean feeding in most applications, and low-oil soybean germplasm would be desirable for that purpose.

In countries such as Austria, a considerable amount of soybean harvested is utilized for soyfood production, as the consumption of soyfoods from GMO-free soybean has increased for a number of reasons. In soyfood production (e.g. soy milk, tofu, soy yoghurts, spreads, desserts, snack products, protein concentrates, meat substitutes etc.), food-grade soybeans are preferred as a starting material which have a yellow seed coat with a light hilum, larger seed size, higher seed protein and sugar content than standard soybean. An elevated seed protein

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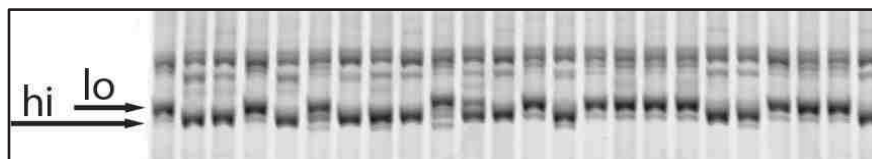


content of at least 42-45% improves the nutritional value of food products, whereas a higher ratio of glycinin (11S) to beta-conglycinin (7S) globulin protein fractions as present in food-grade germplasm is important for characters such as tofu yield or structural properties of products (7). In addition, sucrose is influencing the taste of products and is therefore an important trait for improving consumer acceptance of soyfoods. Food-grade soybean populations reach sucrose contents from 6-8%, whereas conventional or high-protein populations are clearly lower in sucrose content (5).

Genetic variation in food safety characteristics may gain in relevance in the future as soyfood production is increasing. For instance, a reduction of the immunodominant soybean allergen protein P34 (Gly m Bd 30K) through introgression of a null allele is of particular interest, as soybean protein is increasingly utilized in many modern food applications raising the frequency of allergic reactions in sensitive consumers. As soybean production is expanding to new regions of Europe, uptake of heavy metals from contaminated soil is another concern in soyfoods. For cadmium, variation in a single gene coding for an ATPase mediating metal ion transport across cellular membranes in the root has a highly significant effect on seed cadmium concentration (10). A selection procedure based on a microsatellite marker closely associated with the cadmium uptake locus (Fig. 1) can easily be implemented for the identification of low cadmium accumulating genotypes contributing to food and feed safety.

## Genetic diversity

Significant genetic diversity has been described in Central European soybean breeding materials (2) which is essential for further breeding progress. Variation is also available in various seed quality characters in soybean collections which needs to be introgressed into early maturity germplasm (see Fig. 2 for visual inspection of seed diversity). Thus, targeting of soybean quality to European requirements (i.e. protein production) could contribute to establishing domestic soybean production and to increase the range of options for profitable protein crop production in Central Europe. 



**Figure 1. Differentiation between 24 soybean genotypes at the Sack149 SSR marker locus with alleles linked to either high (hi) or low (lo) seed cadmium accumulation (soybean *Cda1* gene)**



**Figure 2. Visual genetic diversity for soybean seed characteristics in conventional and specialty germplasm**

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# Enhancing the economic potential of beans using *Vicia faba* L.: Crop performance and the use of air-classified grain components in animal feeds and brewing

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**Abstract:** Research at the James Hutton Institute's Centre for Sustainable Cropping (CSC) shows biological nitrogen fixation by faba beans (*Vicia faba* L.) is maximal under low input, non-inversion tillage with compost incorporated. Increasing bean protein production efficiency is being investigated with respect to rhizobial diversity and through development of the supply chain. Powdered faba bean kernels are fractionated into bean-protein and -starch concentrates (BPC and BSC, respectively) using 'air-classification'. BPC can displace soya and fishmeal in salmon-feed without impacting fish growth, feed efficiency or health. BSC performed well in poultry- and pig-feeds and has significant socio-economic potential in brewing.

**Key words:** aquaculture, distilling, nitrogen-fixation, protein, rhizobia

we report on: 1) the performance of faba beans (*Vicia faba* L.) with respect to nitrogen fixation and rhizobial diversity under conventional and sustainable agronomic treatments; 2) the efficacy of "air classification", a low cost method which uses a vertical cyclonic air stream to generate bean protein- and bean starch-concentrates (BPC and BSC, respectively; Fig. 1) from milled faba beans (kernels), and 3) the utility of the BPC and BSC in animal production and brewing.

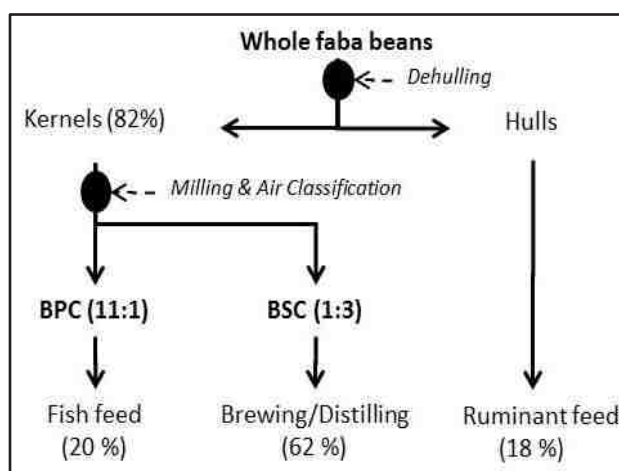
## Materials and methods

Faba bean yield, the proportion of nitrogen (N) derived from air (%Ndfa), biological nitrogen fixation (BNF; 4), and molecular diversity of nodule-associated *Rhizobium leguminosarum* (1, 3, 5, 6) of five bean

varieties were quantified in response to 'conventional' and 'sustainable' growing regimes at the Centre for Sustainable Cropping (CSC; [www.hutton.ac.uk/csc](http://www.hutton.ac.uk/csc)), over three growing seasons (2011-2013). The standardised ileal digestibility (SID) of BSC essential amino acids (AA) was determined in 28 d old birds and 70 d old pigs. Fish growth, feed efficiency and intestinal inflammation were monitored in freshwater Atlantic salmon parr (initial body weight 1.5 g, 18 feeds tested for 56 d) and sea water Atlantic post-smolt salmon (initial body weight 1.276 g, nine feeds tested for 112 d). The potential of whole faba beans (WFB) and BSC was assessed as an adjunct using serial combinations with malted barley grist (WBG) in ale brewing trials at laboratory- and pilot-brewery-scale.

## Introduction

The socioeconomic potential of whole legume grains maybe greatest where yields are high, stable and grains possess the highest possible protein content. In addition, fractionating the grain can increase economic returns by diversifying the range of legume-based products which are available. Any consequent increase in gross margins would encourage greater uptake of legume supported cropped systems and so enhance sustainable production. Towards that end,



**Figure 1. A schematic diagram showing the possible fates of whole faba bean components: including the hulls and bean protein concentrates (BPC) and bean starch concentrate (BSC), derived from the air classified kernels; percentages relate to proportions of whole bean; ratios show the proportion of protein : starch**

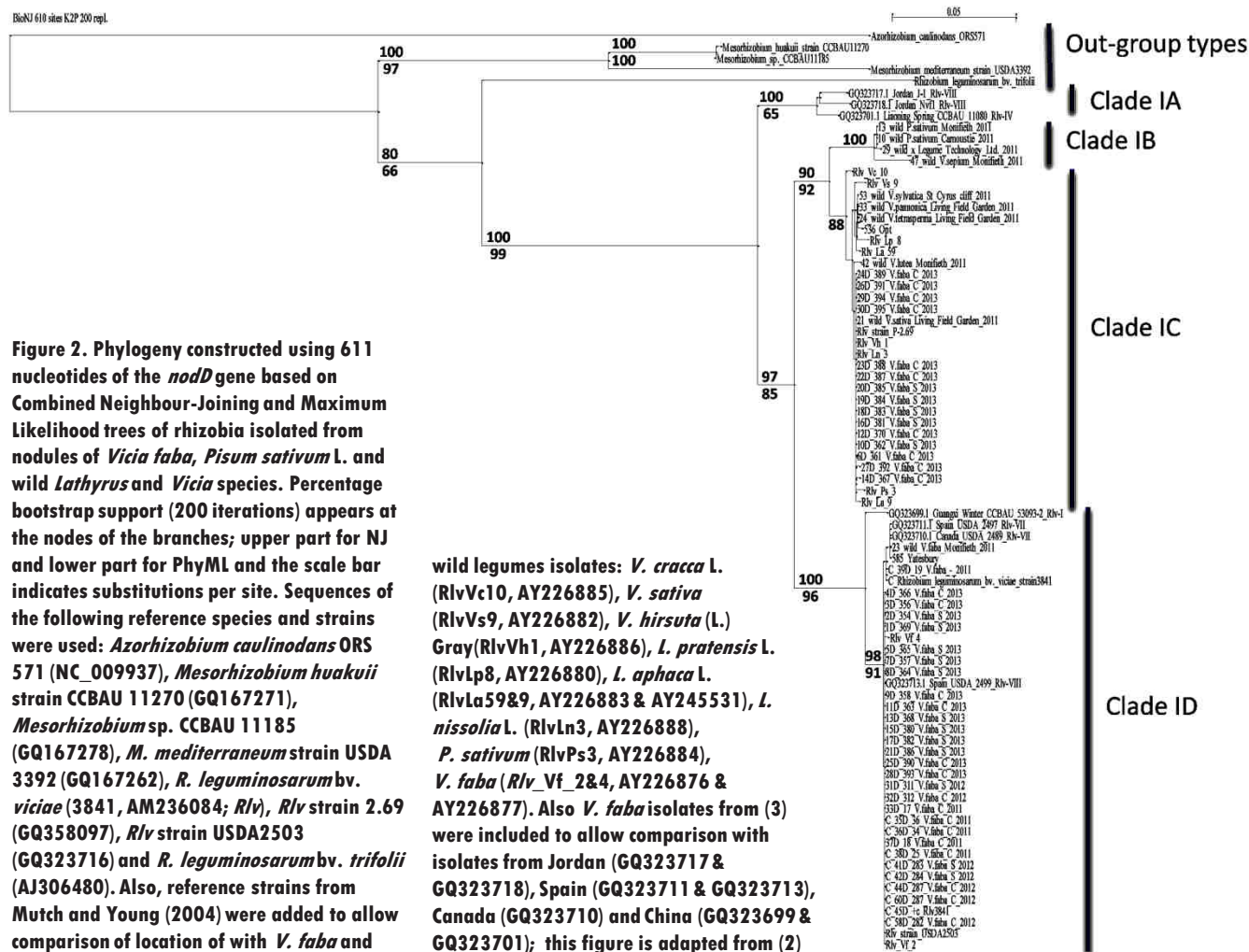
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<sup>5</sup>Inverlmond Brewery, Perth, Scotland, UK



## Results and discussion

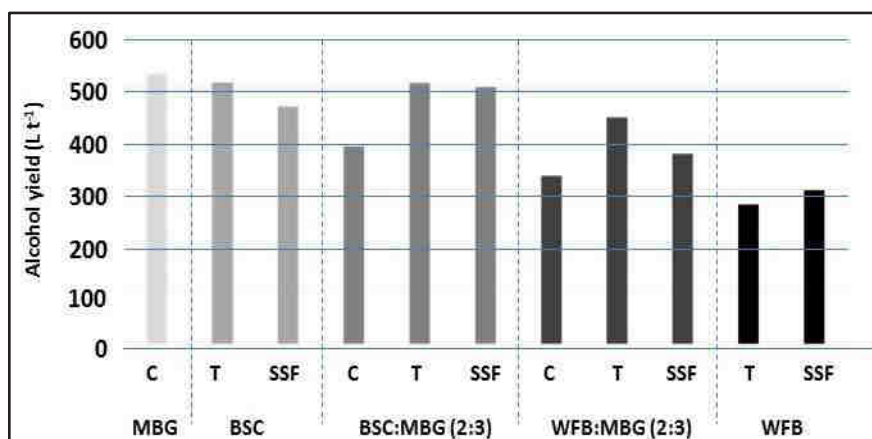
Field data indicated that %Ndfa was highest under sustainable management, with mean %Ndfa values (and %Ndfa increase relative to conventional management) across five cultivars of 87% (+11%) in 2011, 91% (+8%) in 2012 and 87% (+6%) in 2013. BNF was also higher under sustainable treatment with an average of 25 kg ha<sup>-1</sup> more N remaining in the soil compared to conventional practice. Across both treatments, BNF ranged from 150 kg N ha<sup>-1</sup> - 300 kg N ha<sup>-1</sup> with 70% - 90% of the fixed N removed in grain and 25 kg ha<sup>-1</sup> - 80 kg ha<sup>-1</sup> remaining in-field.

Rhizobial diversity studies characterised isolates as distinct from the 'out-group' bacterial types (*Mesorhizobium* and *Azorhizobium*; Fig. 2), and from those isolates known not to nodulate this crop at their diversity centre ('Clade IA'). Thus, the phylogenetic tree showed that rhizobia isolated from faba bean cultivated at the CSC co-localised either with rhizobial types isolated from wild legumes (Clades IB and IC) or *Rlv* (Clade ID). Comparative functional characterisation of the isolates belonging to distinct clades is ongoing.


Salmon feeding studies showed that fish growth was faster or feed conversion more efficient when BPC was included in the feed. Intestinal health of fish was not compromised at levels below 30% inclusion of BPC, indicating the utility of faba bean BPC in salmon feeds.

Pigs and poultry feeding trials showed that the SID of BSC essential AA averaged 84.8 ± 1.6 % and 83.3 ± 2.0 % in pigs and poultry, respectively. Provided its relatively low methionine content is supplemented BSC may be an attractive alternative protein source to reduce reliance on soya bean meal for pigs and poultry.

Brewing trials tested different fermentation methods and enzymatic combinations and identified a novel zymology-based method to produce an ale of 5.0% ABV with good sensory characteristics using 25% [w/w] BSC.



**Figure 3. Alcohol yields of bean starch concentrate (BSC), malted barley grist (MBG) and whole faba bean (WFB) flours alone, or in combination, using three different fermentation systems: conventional (C), using the natural diastatic power of the MBG; traditional (T), using added  $\alpha$ -amylase and glucoamylase in the 'mash', or simultaneous saccharification and fermentation (SSF), using  $\alpha$ -amylase in the mash and added glucoamylase when fermentation is initiated; ratios relate to the respective percentages of wort adjuncts**

The BSC and milled WFB (kernels) can be used alone or in combination with MBG to achieve good alcohol yields (Fig. 3). The three saccharification methods trialed were: conventional (C), employing the natural diastatic power of the MBG; traditional (T), using added  $\alpha$ -amylase and glucoamylase in the 'mash', or; simultaneous saccharification and fermentation (SSF), using  $\alpha$ -amylase in the mash and added glucoamylase when fermentation is initiated -to facilitate the slow and continual release of glucose throughout fermentation. The alcohol yields were highest and similar to MBG-alone when the T and SSF methods were applied to BSC:MBG, or using the T method on BSC-only. Current work aims to identify methods which optimise alcohol production using the BSC and WFB-kernel flours and help develop legume-grain-only (*i.e.* gluten-free) products. See (7), for a full report of this brewing and distilling research: or contact the author. 

#### Acknowledgements

The research reported here is supported by funding from: the Scottish Government, Abertay University, the European Union funded project Legume Futures ([www.legumefutures.eu](http://www.legumefutures.eu), [www.elrc.eu](http://www.elrc.eu)) and the Technology Strategy Board ([www.beans4feeds.net](http://www.beans4feeds.net), TSB101096). The industrial partners of beans4feeds are EWOS Ltd., BioMar Ltd., Limagrain UK Ltd., Marine Harvest (Scotland) Ltd. and Harbro Ltd. The academic partners are the Universities of Stirling, Aberdeen, St. Andrews, Scotland's Rural Universities College and the James Hutton Institute.

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# Reducing use of fossil energy by biological N fixation

by Hannu KÄNKÄNEN

**Abstract:** Biological nitrogen (N) fixation can be increased greatly in Finnish agriculture. Intensive use of legumes in grasslands, utilization of green manure and undersown crops, and maximal production of pulse crops could reduce use of fertilizer N by 60 % compared to current situation. It would reduce the usage of fossil energy, taking into account the energy use of machines, by about 3,700 TJ per year. This corresponds to an energy amount of the fuel oil which was used for grain dryers and heating of farm buildings in Finnish farms in 2010.

**Key words:** fertilizer, fossil energy, green manure, legumes, nitrogen

Non-legumes can be replaced with legumes in various ways. The area of pulses could be tenfold in Finnish agriculture (Fig. 1), which would mean about a 60 kg ha<sup>-1</sup> decrease in use of inorganic N compared to cultivation of cereals. The greatest potential is however in silage production, where clovers or alfalfa could be grown in mixtures with grasses on almost half a million hectares. Compared to pure grasses, this would save 110 kg inorganic N ha<sup>-1</sup> year<sup>-1</sup>. Further, increased usage of undersown clovers and green manures could decrease the use of inorganic N at arable farms.

## N for subsequent crop

Knowledge concerning legume crops which are able to replace fertilizer N in different cropping situations was gathered. N fertilization replacement value (NFR) (2) of the legume crop is estimated as the amount of chemical fertilizer that could be reduced after growth of legumes, without reduction in the optimal growth of the subsequent non-legume crop. NFR for clover-grasses was estimated, according to e.g. (7) and (8), to be about 30 kg ha<sup>-1</sup> and for pulses (according to literature and own experiments) 25 kg ha<sup>-1</sup>. Growth and N yield of undersown clovers vary a lot, but as an average NFR for them is 20 kg ha<sup>-1</sup> (4).

## Great but realistic potential

The potential of biological N fixation (BNF) for reducing the usage of fossil energy was estimated on the basis of current field area of different crops in Finland. The available manure N was taken into account, but not the possible increased efficiency in its usage in the future. Field use was supposed to change only in the context of increasing BNF, not e.g. because of increased use of fields for energy crops. The possibilities of legumes were estimated optimistically, but restrictions such as adequate crop rotation and soil type were taken into account.

European production of ammonia (NH<sub>3</sub>), of which N fertilizers are almost entirely manufactured, consumes on average 35.2 GJ t<sup>-1</sup> of fossil energy per ton (10). However, our calculations were based on 31.8 GJ t<sup>-1</sup> of NH<sub>3</sub> which is the value for the most effective techniques in current factories. Based on this, the total energy consumption of fertilizer N production and transportation is about 39 GJ t<sup>-1</sup>.



**Figure 1.** The area of grain legumes could be tenfold in Finnish agriculture

**Table 1.** Nitrogen (N) benefit after one growing season with hairy vetch and red clover for succeeding spring barley, when the succeeding barley was fertilized with 0 kg N ha<sup>-1</sup>, 40 kg N ha<sup>-1</sup> or 80 kg N ha<sup>-1</sup>; values are based on grain N (kg ha<sup>-1</sup>) yields in comparison to barley after barley with 80 N at six experimental sites

Crop	Hairy vetch			Red clover		
	0 N	40 N	80 N	0 N	40 N	80 N
Fertilizer N for barley						
N benefit (kg ha <sup>-1</sup> ) after green manure						
Early autumn ploughing	75	42	19	57	28	-3
Late autumn ploughing	74	49	18	62	37	7
Spring ploughing	72	48	14	55	33	1
Spring reduced tillage	69	49	17	53	25	1
Average	73	47	17	57	31	2

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The N benefit for the subsequent crop after hairy vetch (*Vicia villosa* Roth) and red clover (*Trifolium pratense* L.) green manure crops was computed here in a new way, using old data from field trials carried out and published already in the 1990's (5, 6). NFR was now determined based on average after effects of six experimental sites and with help of three fertilizer N levels for the succeeding crop. In these experiments, vetch, clover and straw residues of spring barley (*Hordeum vulgare* L.) were incorporated into the soil by ploughing in early autumn (beginning of September), late autumn (end of October) and spring (May) and by reduced tillage in spring. Vetch and clover were grown as annual, spring-sown green fallows. Spring barley was sown as the succeeding crop in May and fertilized with 0 N ha<sup>-1</sup>, 40 kg and 80 kg N ha<sup>-1</sup> after legumes and with 40 kg N ha<sup>-1</sup>, 80 kg N ha<sup>-1</sup> and 120 kg N ha<sup>-1</sup> after barley.

Different methods to determine NFR were tested. Comparing yields of 0 N, 40 N and 80 N fertilized barley after legumes with normally (80 N) fertilized barley after barley was found to be the most reliable method. The after effect of vetch corresponded to more than 70 kg ha<sup>-1</sup> or less than 20 kg ha<sup>-1</sup> fertilizer N, when 0 N or 80 N was given to barley, respectively (Table 1). In other words, the high N yield of vetch is utilized ineffectively, if high amount of fertilizer N is given.


The smaller N yield of red clover resulted in lower N benefit compared to that of vetch (Table 1). In fact, if any replace of fertilizer N is expected after one growing season with red clover, the amount of added fertilizer N has to be under the biological optimum. Establishing red clover already in the previous spring as an undersown crop can lead to much higher N yield (Fig. 2).

As a conclusion of the calculations, NFR of well grown green manure crop with high legume content was set to be 70 kg ha<sup>-1</sup>. Further, after perennial green fallow, NFR of 20 kg ha<sup>-1</sup> for the second after-effect year was expected, based on the assumption that N release continues after the first year (1). On the other hand, sometimes the biological and possibly even economical optimum must be turned down, if N in green manure is wanted to be used maximally by the subsequent crop.

## Many advantages, lot of uncertainties

The maximal use of BNF probably has positive effects on farm economy in a long run. According to the newest literature, profitability of farming is higher with than without legumes (9). Further, for instance grass-based production of biogas could still improve the farm profitability. Fertilizers as secondary products from bioenergy generation should also be studied further.

Although great lack in knowledge concerning effects on environment occurs, it seems obvious that increased use of legumes would improve the carbon content (3) and structure of soils. Decreasing production of synthetic fertilizers would diminish greenhouse gas emissions from industry, but effects of different plant materials and cultivation techniques on emissions from agriculture need research. Management of the legume N needs further studies to keep leaching of nutrients at levels as small as possible. Further, many aspects concerning practices and economy in legume usage would be worth careful examination.

Our results show that it is possible to markedly decrease the use of synthetically produced fertilizer N, and thus consumption of fossil energy, with help of BNF. Intensive use of legumes could reduce use of fertilizer N by 60 % compared to current situation in Finnish agriculture. It would reduce usage of fossil energy by about 3700 TJ year<sup>-1</sup>. Policies aiming at energy saving could support the change in cultivation practises. Motivation of farmers has a great influence, when farming systems are improved comprehensively. It is increased by policies and the concern about soil fertility and desired reduction in use of expensive inorganic fertilizers. 



**Figure 2. When red clover is established as an undersown crop in the previous spring cereal crop, it can produce a high N yield already in the first actual green fallow summer**

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# All legumes are beautiful, but some legumes are more beautiful than others

by Aleksandar MIKIĆ

**Abstract:** Although one is often is impressed by any legume's beauty while in bloom, it is easily forgotten in favour of other economically more important uses. Making this world more beautiful depends solely on us and breeding and cultivating various legumes for beauty itself is surely one of the ways to fulfil this noble task. The article presents selected ornamental grown and wild legume species.

**Key words:** beauty, legumes, ornamental plants

## Introduction

Apart from their prominent roles as food and feed, legumes have numerous forms of non-food uses, with green manure and biofuel as the most widely present in agriculture and industry. Most legume species are easily recognised for the colour of their flowers and it is rather natural there are those grown solely for ornamental purposes.

## Fabeae and Trifolieae

One of the most renown decorative legume species is sweet pea (*Lathyrus odoratus* L.), an annual with great diversity of flower size and petal shape (Fig. 1). Breeding sweet pea has a tradition long more than a century, especially in UK, resulting in developing a large number of sweet pea cultivars of diverse flower colour, size and shape and fragrance length and intensity. The largest collection of sweet pea in the world was established and is maintained by Roger Parsons, gaining a status of a UK national collection and comprising about 900 cultivars of sweet pea and around 100 taxa of other *Lathyrus* species (3).

Apart from cultivated species, the tribe Fabeae is extremely rich in wild species of an extraordinary beauty. It is simply not possible to mention all, but could be enough to select large-flowered vetchling (*Lathyrus grandiflorus* Sm.), 'beautiful' vavilovia (*Vavilovia formosa* (Stev.) Fed.) and large-flowered vetch (*Vicia grandiflora* Scop.) (Fig. 1).

Although red (*Trifolium pratense* L.) and white (*T. repens* L.) clovers are surely among the most decorative crops of the tribe Trifolieae, the species such as crimson (*T. incarnatum* L.) and blushing (*Trifolium rubens* L.) clovers, when in bloom, provide an ordinary field intended for forage production or green manure with a surreal scenery (Fig. 1).



**Figure 1. Some Fabeae and Trifolieae of exceptional beauty: (above, left) sweet pea, (above, right) large-flowered vetch, (middle, left) 'beautiful' vavilovia, (middle, right) white clover, (below, left) crimson clover and (below, right) blushing clover**

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**Figure 2. Some ornamental legume species:** (first row, left) garden lupin, (first row, right) Texas Bluebonnet, (second row, left) hyacinth bean, (second row, right) Japanese wisteria, (third row, left) Persian silk tree, (third row, right) Judas tree, (fourth row, left) honey locust, (fourth row, right) golden-chain tree, (fifth row, left) black locust and (fifth row, right) Pagoda Tree

### Lupins and hyacinth bean

Garden lupin (*Lupinus polyphyllus* Lindl.) is a perennial herbaceous species native to North America (3). The most significant breeding efforts were made by George Russell from UK, developing an ideotype present in majority of the contemporary cultivars. Texas Bluebonnet (*Lupinus texensis* Hook.), is the favourite of many Texans and one of the state flowers of Texas.

Hyacinth bean (*Lablab purpureus* (L.) Sweet) may be seen in the gardens across Europe, with breeding programmes aimed at flower number and size and number of pods.

### Woody perennial legumes

In this group, the most familiar are Japanese wisteria (*Wisteria floribunda* (Willd.) DC.), a liana species, and deciduous trees, such as Persian silk tree (*Albizia julibrissin* Durazz.) Judas tree (*Cercis siliquastrum* L.), honey locust (*Gleditsia triacanthos* L.), golden-chain tree (*Laburnum × watereri* (G. Kirchn.) Dippel) and Pagoda Tree (*Styphnolobium japonicum* (L.) Schott). Breeding programmes are aimed at tree growing habit, time and length of flowering, flower scent and stress resistance (2).

### Acknowledgements

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# Lucerne and clover in pure stand or in association with ryegrasses under intensive conditions in Flanders

by Alex DE VliegHER and Gerda CNOPS

**Abstract:** Monocultures of red (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.), lucerne, perennial (*Lolium perenne* L.) and Italian ryegrass (*Lolium multiflorum* Lam.) and grass/legume mixtures were compared at 3 levels of chemical N fertilisation: 0 kg N ha<sup>-1</sup>, 105 kg N ha<sup>-1</sup> and 265 kg N ha<sup>-1</sup> in order to investigate the effect on botanical composition, dry matter yield, forage quality and nitrate residue in the soil at the end of the growing season. From agricultural point of view, mixtures of ryegrass with clover or lucerne, fertilised with 105 N ha<sup>-1</sup> were the best in the 4-year period (2004-2007). The levels of nitrate residue in the soil were very acceptable for all the treatments and were the highest for the legume monocultures and the lowest for the grasses.

**Key words:** clover, *Lolium*, lucerne, nitrate quality, residue, yield

Dairy farming in Flanders, the northern part of Belgium, is very intensive: specialised dairy farms have on average 100 cow units with 2.24 animals ha<sup>-1</sup>, an average milk production of 7100 l cow<sup>-1</sup> and 1.81 full time workers (farmer + family) farm<sup>-1</sup>. Grass and silage maize (*Zea mays* L.) are the dominant

forages and lucerne (*Medicago sativa* L.) is really a crop of minor importance at this moment. However, there are many reasons to renew the interest for this protein crop: (i) the restriction in nitrogen (N) use on grassland to implement the EU- nitrate guidelines restricts the crude protein (CP) concentration and yield ha<sup>-1</sup>, in a grass/maize silage ration supplementation with expensive soya meal is needed, cost price of chemical N-fertilisers is high, subsidies in the frame of rural development are available and common agricultural policy (CAP) reform 2015 stimulates growing protein crops.

## Materials and methods

The experiment was carried out in Merelbeke, with sandy loam soil, pH 6.1, altitude 11 m, average annual temperature 9.9°C, average annual precipitation 780 mm.

Monocultures of red clover (*Trifolium pratense* L., Tp) and white clover (*Trifolium repens* L., Tr), lucerne, perennial ryegrass (*Lolium perenne* L., Lp) and Italian ryegrass (*Lolium multiflorum* Lam., Lm) and 7 grass/legume mixtures were established as a complete block design with 3 replicates in April 2003 at recommended seeding rates (Fig. 1). The proportion of Ms, Tp and Tr in the grass/legume mixtures was 40%, 30% and 10% respectively.



**Figure 1.** The field experiment with the effect of N fertilisation on forage quality of grass/legume mixtures and nitrate residue in the soil, comprising *Medicago sativa*, *Trifolium pratense*, *T. repens*, *Lolium multiflorum* and *L. perenne*, in Merelbeke, Flanders, Belgium from 2003 to 2007

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**Table 1. Effect of N fertilisation on botanical composition and dry matter yield of grass/legume mixtures, average of 2004-2007; Ms: *Medicago sativa*, Tp: *Trifolium pratense*, Tr: *T. repens*, Lm: *Lolium multiflorum*, Lp: *L. perenne***

Treatment	Content of sown legumes % in dry matter (DM)			DM yield (Mg ha <sup>-1</sup> year <sup>-1</sup> )			DM yield (kg kg <sup>-1</sup> fertiliser N)	
	N0	N105	N265	N0	N105	N265	N105	N265
Lm				5.93	9.90	11.82	37.8	22.2
Lp				4.73	7.88	10.90	30.0	23.3
Tp				12.88	13.24	13.84	9.1	3.6
Tr				9.06	9.44	9.61	3.6	2.1
Ms				15.11	15.84	17.14	7.0	7.7
Tp + Lm	67	55	33	13.30	14.38	14.77	10.3	5.5
Tp + Lp	76	64	54	13.32	14.16	14.66	8.0	5.1
Tp + Tr + Lp	51 + 23	46 + 22	36 + 20	12.93	14.08	15.01	11.6	7.8
Tr + Lp	50	50	33	12.04	13.46	14.01	11.0	7.4
Ms + Lm	80	57	58	15.65	17.11	17.77	13.9	8.0
Ms + Lp	87	77	74	16.26	16.69	17.21	4.1	3.6
Ms + Tr + Lp	81 + 7	75 + 6	66 + 12	15.97	17.05	17.29	10.3	5.0
Mean								
Grasses				5.33	8.89	11.36	33.9	22.8
Legumes				12.35	12.84	13.53	4.7	4.5
Grass/legumes	75	65	55	14.21	15.27	15.82	10.1	6.1

**Table 2. Effect of N fertilisation on forage quality of grass/legume mixtures and nitrate residue in the soil, average of 2004-2007; Ms: *Medicago sativa*, Tp: *Trifolium pratense*, Tr: *T. repens*, Lm: *Lolium multiflorum*, Lp: *L. perenne***

Treatment	Energy content			Protein content						NO <sub>3</sub> -N in the soil		
	VEM (kg <sup>-1</sup> DM)			DVE (g kg <sup>-1</sup> DM)			OEB (g kg <sup>-1</sup> DM)			(kg ha <sup>-1</sup> )		
	N0	N105	N265	N0	N105	N265	N0	N105	N265	N0	N105	N265
Lm	832	825	788	68	66	66	-17	-15	-5			
Lp	837	837	829	70	71	71	-7	-6	-4	6	8	8
Tp	731	740	743	88	89	90	61	61	60	16	17	17
Tr	852	849	852	109	109	109	66	66	65	21	19	34
Ms	703	699	697	82	80	80	54	51	51	15	15	16
Tp + Lm	754	765	772	80	75	74	43	25	24			
Tp + Lp	748	759	764	84	84	82	49	48	44	8	19	16
Tp + Tr + Lp	779	764	780	89	87	84	49	47	46			
Tr + Lp	829	810	811	91	85	83	42	36	31	9	14	13
Ms + Lm	716	719	717	78	76	73	46	39	39			
Ms + Lp	718	721	720	81	79	81	51	43	50	12	12	14
Ms + Tr + Lp	740	735	731	86	83	82	53	50	51			
Mean												
Grasses	835	831	809	69	69	69	-12	-10	-5	6	8	8
Legumes	762	763	764	93	93	93	93	59	59	17	17	22
Grass/legumes	755	755	756	84	81	80	80	41	35	10	15	14

Three levels of chemical N fertilisation were established for all the treatments: 0 kg N ha<sup>-1</sup> y<sup>-1</sup>, 105 kg N ha<sup>-1</sup> y<sup>-1</sup> and 265 kg N ha<sup>-1</sup> y<sup>-1</sup>, hereafter designated N0, N105 and N265. The highest N-level corresponded to the maximum level of active N that can be applied in accordance with the Flemish manure action plan (MAP III). Nitrogen of N 105 was applied for the first (52.5 N) and the second cut (52.5 N). Nitrogen of N265 was divided over the first 3 cuts: 90 kg N ha<sup>-1</sup> y<sup>-1</sup> - 90 kg N ha<sup>-1</sup> y<sup>-1</sup> - 85 kg N ha<sup>-1</sup> y<sup>-1</sup>. All treatments received 60 kg P<sub>2</sub>O<sub>5</sub> and 360 K<sub>2</sub>O ha<sup>-1</sup> y<sup>-1</sup>.

The plots (12 m<sup>2</sup> gross area, 8.4 m<sup>2</sup> net area) were cut 3 times in the first year and 5 times in the following years. At each cut dry matter (DM) yield was measured and a grab subsample was separated into the individual sown species and an unsown part. Samples were analysed by NIRS to determine chemical composition and digestibility and energy (fodder unit milk - VEM) and protein content (true protein digested in the small intestine - DVE and rumen degraded protein balance - OEB) were calculated. The nitrate nitrogen content of the 0-90 cm top soil was determined yearly in the beginning of November).

## Results and discussion

The proportion of weeds (= unsown species) in the total DM yield in the period 2004-2007 was very low for grass/legume mixtures (<2%) and Tp (2%), low for Ms (<<10%) and Lm (<<10%) and relatively important for Tr (<10%) and Lp (<15%). In the monoculture of the ryegrasses white clover was the main intruder and in the monocultures of the legumes *Lolium* species and *Poa annua* L. were the main invasive species. In the monocultures of grasses weeds significantly decreased with fertilisation while weeds tended to increase with fertilisation in the legume monocultures. There was a tendency of a decrease in invasion by white clover when more N was used in the grass treatments.

In the monocultures N105 and N265 resulted respectively in an additional yield of 0.49 Mg DM ha<sup>-1</sup> and 1.18 Mg DM ha<sup>-1</sup> or 4.7 kg DM kg<sup>-1</sup> and 4.5 kg DM kg<sup>-1</sup> fertiliser N in comparison with N0 (Table 1).

The proportion of legumes in the grass/legume mixtures significantly decreased when more N was applied but the level of legumes was always substantial, especially for the combinations with Lp in N105. For grass/legume mixtures, N105 and N265 resulted in an increase of the DM yield by 1.06 Mg ha<sup>-1</sup> and 1.61 Mg ha<sup>-1</sup> respectively. It is obvious that the small difference in DM yield between these two N levels (0.55 Mg ha<sup>-1</sup>) did not economically justify the extra use of 160 kg N on grass/legume mixtures. The use of 105 kg N ha<sup>-1</sup> resulted in an additional yield of 10.1 kg DM kg<sup>-1</sup>N which corresponded with an cost price of 0.10 € kg<sup>-1</sup> DM (765 € Mg<sup>-1</sup> N for fertiliser and 20 € fertiliser application<sup>-1</sup> ha<sup>-1</sup>).


The growth of Lp and Lm is limited by a N fertilisation of 265 kg N<sub>available</sub> ha<sup>-1</sup> (1, Table 1) but the MAP III regulation did not allow to apply more N. The MAP IV regulation (2011-2014) allows to apply 300N<sub>available</sub> ha<sup>-1</sup> (sandy soils).

In this experiment there is no correlation between the energy content of the dry matter and the level of N fertilisation (Table 2). Tr, followed by Lp, Lm and Tr + Lp had the highest energy content (> 800 VEM) and Ms (700 VEM) as well as grass/lucerne mixtures (716-740 VEM) the lowest. One can expect that a higher proportion of grasses in the mixture, caused by a higher N use, should increase the energy content of the dry matter but this was not observed in this experiment. The protein content, in terms of DVE and OEB, was more affected by type of monoculture or mixture than applied N-level. The pure stands of the legumes Tr had the highest level of protein in the DM and the monocultures of ryegrasses, handicapped by the inferior level of N fertilisation, had significant lower protein levels than the mixtures. The average DVE content of the grass/legume mixtures was 81 g kg<sup>-1</sup> DM, which is comparable to perennial ryegrass in favourable growing conditions. The mixtures with Lm had a significant smaller DVE protein content in comparison with the mixtures with Lp. This is in accordance with previous observations (2).

To avoid leaching, the nitrate nitrogen content in the soil profile 0 cm - 90 cm should not exceed 90 kg ha<sup>-1</sup> at the end of the growing season. The nitrate residue in the soil was relatively higher for the monocultures of legumes (17 kg N ha<sup>-1</sup> - 22 kg N ha<sup>-1</sup>) in comparison with grass/legume mixtures (10 kg N ha<sup>-1</sup> - 15 kg N ha<sup>-1</sup>) and grasses (6 kg N ha<sup>-1</sup> - 8 kg N ha<sup>-1</sup>) but they were always far below the legal limit of 90 kg ha<sup>-1</sup> (Table 2). There was no effect of N fertilisation on the nitrate residue in the soil.

## Conclusions

The use of mineral N fertilisers at up to 265 kg N ha<sup>-1</sup> had no influence on the legume content and on the DM yield (except for the N265 treatment of lucerne) in monocultures of red or white clover or lucerne. The proportion of legumes in the grass/legume mixtures decreased when more N was used but the level of legumes remained substantial. For the mixtures, the use of 105 kg N ha<sup>-1</sup> y<sup>-1</sup> or 265 kg N ha<sup>-1</sup> y<sup>-1</sup> resulted in an additional yield of about 1.1 kg N ha<sup>-1</sup> y<sup>-1</sup> and 1.6 Mg ha<sup>-1</sup>, respectively. No systematic correlation was found between the energy or protein content of the dry matter and the level of N fertilisation. Growing legumes or grass/legume mixtures under a cutting regime gave no difficulties in terms of nitrate residue.

Grass/legume mixtures with a rather low N fertiliser input (e.g. 105N<sub>available</sub> ha<sup>-1</sup> by slurry + some chemical fertilisers) have a lot of potential: a higher DM yield in comparison with the components in pure stand, an higher energy content than legumes in pure stand (except white clover), a higher protein content in comparison with ryegrasses (265N<sub>available</sub> ha<sup>-1</sup>) and no difficulties in terms of nitrate residue. 

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# Intercropping annual legumes and brassicas for forage production

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**Abstract:** The available literature on intercropping brassicas with legumes is rather scarce, although there are recently published results showing diverse benefits for a brassica component, where a legume component assists its brassica companion in uptaking less available nutrients much easier. A series of field trials was aimed at assessing the potential of intercropping various autumn- and spring-sown brassicas with legumes for forage production. This scheme proved beneficial for both, enabling the brassica component to bring forth its full potential, providing the legume with support and using the available stand space much better. **Key words:** brassicas, forage yield, intercropping, land equivalent ratio, legumes

Two brassicas, fodder kale (*Brassica oleracea* L. var. *viridis* L.) cv. Perast and rapeseed (*Brassica napus* L. var. *napus*) cv. Zorica, played the role of supporting crops for four legumes, namely pea (*Pisum sativum* L.) cv. NS Krmni, common vetch (*Vicia sativa* L.) cv. NS Tisa, Hungarian vetch (*V. pannonica* Crantz) cv. Panonka and hairy vetch (*V. villosa* Roth) cv. NS Viloza, acting as supported crops, according to a specific legume-brassica intercropping scheme (Fig. 1). All six cultivars, were also sown as sole crops. In all six pure stands and their eight intercrops, forage dry matter yield ( $\text{t ha}^{-1}$ ) and its land equivalent ratio for forage dry matter yield ( $\text{LER}_{\text{FDMY}}$ ) were determined.

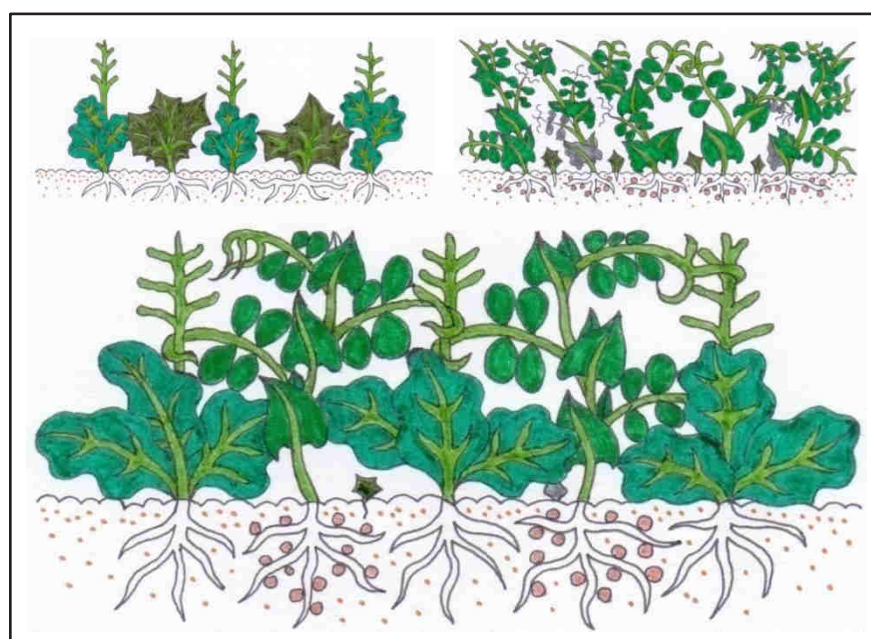
The highest two-year average individual contribution in the total forage dry matter yield among brassicas was in fodder kale ( $4.5 \text{ t ha}^{-1}$ ) when intercropped with Hungarian vetch (Table 1). The highest individual contribution in the total forage dry matter yield among legumes was in hairy vetch ( $6.6 \text{ t ha}^{-1}$ ) when intercropped with rapeseed. The two-year average values of  $\text{LER}_{\text{FDMY}}$  ranged between 1.05 in the intercrop of fodder kale with common vetch and 1.14 in the intercrop of fodder kale with Hungarian vetch. The autumn-sown intercrops of brassicas with legumes have demonstrated a considerable potential for forage production.

## Introduction

Little is known on intercropping brassicas with legumes, although certain recent results demonstrate multiple benefits for a brassica component, especially in terms of easier uptake of less available nutrients due to a positive influence of its legume companion.

## Autumn-sown intercrops

Two small-plot trial has been carried out in the trial years of 2010/2011 and 2011/2012 at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi in the vicinity of Novi Sad.



**Figure 1. A model of intercropping brassicas with legumes: (top, left) often sown in wide rows, brassicas suffer from heavy weed infestations; (top, right) forage legumes, such as pea, fight the weeds easily, but are rather susceptible to lodging that results in partial or complete withering and loss of lower leaves; (bottom) intercropping brassicas with legumes is beneficial for both, enabling the brassica component to bring forth its full potential and providing the legume with support**

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**Table 1. Two-year average values of forage dry matter yield ( $t\ ha^{-1}$ ) and its land equivalent ratio ( $LER_{FDMY}$ ) in pure stands and intercrops of autumn-sown brassicas and legumes at Rimski Šančevi for 2010/2011 and 2011/2012**

Pure stand / Intercrop	Brassica forage dry matter yield	Legume forage dry matter yield	Total forage dry matter yield	$LER_{FDMY}$
Fodder kale	7.5	-	-	-
Rapeseed	6.9	-	-	-
Pea	-	9.2	-	-
Common vetch	-	8.5	-	-
Hungarian vetch	-	6.5	-	-
Hairy vetch	-	9.6	-	-
Fodder kale + pea	4.0	4.8	8.8	1.06
Fodder kale + common vetch	4.2	4.2	8.4	1.05
Fodder kale + Hungarian vetch	4.5	3.5	8.0	1.14
Fodder kale + hairy vetch	3.2	6.1	9.3	1.06
Rapeseed + pea	3.7	5.2	8.9	1.10
Rapeseed + common vetch	3.8	4.5	8.3	1.08
Rapeseed + Hungarian vetch	4.1	3.3	7.4	1.10
Rapeseed + hairy vetch	2.8	6.6	9.4	1.09
$LSD_{0.05}$	0.5	0.9	0.8	0.03

**Table 2. Two-year average values of forage dry matter yield ( $t\ ha^{-1}$ ) and its land equivalent ratio ( $LER_{FDMY}$ ) in pure stands and intercrops of spring-sown brassicas and legumes at Rimski Šančevi for 2011 and 2012**


Pure stand / Intercrop	Brassica forage dry matter yield	Legume forage dry matter yield	Total forage dry matter yield	$LER_{FDMY}$
Rapeseed	6.9	-	-	-
White mustard	4.2	-	-	-
Pea	-	8.4	-	-
Common vetch	-	7.6	-	-
Grass pea	-	8.8	-	-
Rapeseed + pea	3.7	4.9	8.6	1.12
Rapeseed + common vetch	3.9	4.2	8.1	1.12
Rapeseed + grass pea	3.1	5.5	8.6	1.07
White mustard + pea	2.5	5.5	8.0	1.25
White mustard + common vetch	2.7	4.2	6.9	1.20
White mustard + grass pea	1.8	6.2	8.0	1.13
$LSD_{0.05}$	0.4	0.8	0.8	0.09

## Spring-sown intercrops

The second trial included six intercrops of spring-sown brassicas with spring-sown annual legumes. Rapeseed cv. Jovana and white mustard (*Sinapis alba* L.) cv. NS Gorica played the role of supporting crops for pea cv. Jantar, common vetch cv. Perla, and grass pea (*Lathyrus sativus* L.), acting as supported crops. All five cultivars were also sown as sole crops.

The two-year average forage dry matter yield in the sole crop of rapeseed ( $6.9\ t\ ha^{-1}$ ), while the two-year average forage dry matter yield in the sole crop of pea was also higher than in the preliminary results. The highest two-year average individual contribution in the total forage dry matter yield among brassicas was in rapeseed ( $3.9\ t\ ha^{-1}$ ) when intercropped with common vetch, while the highest individual contribution in the total forage dry matter yield among legumes was in grass pea ( $6.2\ t\ ha^{-1}$ ) when intercropped with white mustard. The two-year average values of  $LER_{FDMY}$  ranged between 1.07 in the intercrop of rapeseed with grass pea and 1.25 in the intercrop of white mustard with pea.

## Conclusions

Despite its preliminary character, the results of the trials with intercropping brassicas with legumes show they have a great potential for forage production and thus deserve more attention. Most of the tested intercrops are notable for a balance between the single contribution of each component to the total forage dry matter yield. Among the future steps of this research are physiological, quality and microbiological aspects. 

## Acknowledgements

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# Seed production of white and red clover

by Birte BOELT

**Abstract:** In order to benefit from the improvements brought about by modern plant breeding, high-quality seeds from the improved varieties must be commercially available to farmers. Increasing ovule and floral fertility have the potential to improve seed yield without compromising the vegetative development in forage legumes. In the insect pollinated species white (*Trifolium repens* L.) and red (*T. repens*) clover, the availability of strong honeybee families and native pollinators are required to obtain high seed yields. Improving our knowledge on the interaction between the seed crop and the pollinating insects may help increase ovule and floral fertility and hence seed yield.

**Key words:** ovule fertility, pollination, red clover, reproductive development, white clover

## Producing high seed yields in forage legumes

In many countries, new forage varieties are tested for their Value for Cultivation and Use (VCU), which as a minimum includes dry matter yield and persistence data. VCU testing motivates a constant yield improvement, and an annual increase of 0.3% - 0.5% in dry matter yield of forage legume cultivars has been reported (4). Breeding efforts are primarily related to the vegetative development; however, with the increasing demand for plant products for food, feed and bioenergy a high seed yield potential is required to allow for the commercial success of new forage legume varieties.

In seed production of white and red clover (Fig. 1), crop management techniques are applied to stimulate reproductive development in order to obtain high seed yields. These include establishment at low plant density, manipulation of canopy size to avoid lodging and shading of fruiting organs, synchronization of flowering with pollinating insects as well as controlling pests. High seed yield is correlated to inflorescence density and seed yield per inflorescence, traits for which moderate to high heritability have been found.

## Seed yield components

Inflorescences form in the leaf axils, and commonly, an inflorescence forms at the base of every third leaf produced (8). In average each inflorescence has 60-70 florets with an ovary containing six ovules (5). Each ovule is capable of developing into a mature seed after successful pollination by bees, but on average only 2-3 seeds develop per pod (Table 1, Fig. 2). In red clover seed production experiments, 90-110 florets per

flowerhead were recorded (7). The ovary normally contains two ovules, but usually only one develops into a seed (5). Outcrossing species generally have a low seed/ovule ratio (9).

## Seed yield improvements

Increasing ovule and floral fertility have the potential to improve seed yield without compromising the vegetative development of forage legumes. White and red clover are insect-pollinated species. In white clover the allocation of strong honeybee families and favourable weather conditions will allow successful pollination. In red clover, particularly in tetraploid varieties, the long corolla tube is considered a constraint for successful pollination by honeybees - except for florets with large amounts of nectar. Instead bumblebees, particularly the long-tongued species, are preferred for pollination of red clover (Fig. 3). But in agricultural areas with intensively utilized cropping systems the number of bumblebees and other native pollinators is declining due to a lack of nesting sites (3).



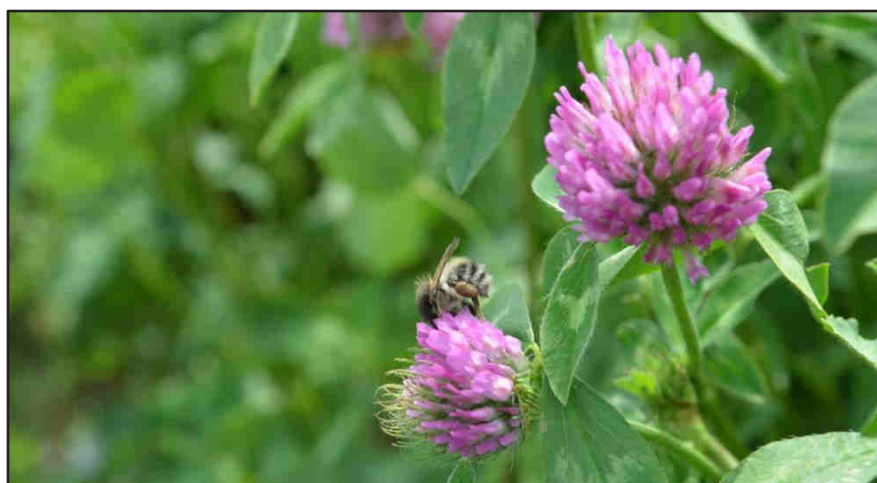
Figure 1. The major seed producing regions of white and red clover in the world (in red colour)

**Table 1. Average values of individual seed yield components and potential seed yield in white clover recorded in Danish seed production experiments (1, 2, 6)**


Number of mature seed heads (m <sup>-2</sup> )	800-1000
Number of flowers per seed head	60-70
Number of seeds per pod	2-3
Seed weight (mg)	0.5-0.7
Potential seed yield (kg ha <sup>-1</sup> )	480-1470



**Figure 2. The number of seeds per red clover flowerhead is currently being monitored in local seed production sites in Denmark**



**Figure 3. *Bombus pascuorum* in a red clover seed crop**

There is an increasing interest to investigate the interaction between the seed crop and the pollinating insects with the focus to identify factors which can improve the pollination efficiency and hence seed yield in white and red clover. 

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# Ascochyta blight resistance in cool season food legumes

by Diego RUBIALES

**Abstract:** Cultivation of most cool season food legumes is hampered by the occurrence of ascochyta blights. Genetic variation for resistance has been reported in most crops and is being used by breeders. However, available resistances use to be incomplete and of complex inheritance controlled quantitatively by multiple genes. Genetic studies have resulted in the identification of a number of QTLs in most crops, but progress in delivery of markers usable in MAS has been slow. This, together with the insufficient understanding on the variability of the pathogen populations has complicated resistance breeding. Current achievements and prospects in breeding for resistance to ascochyta blights in major cool season food legumes will be discussed.

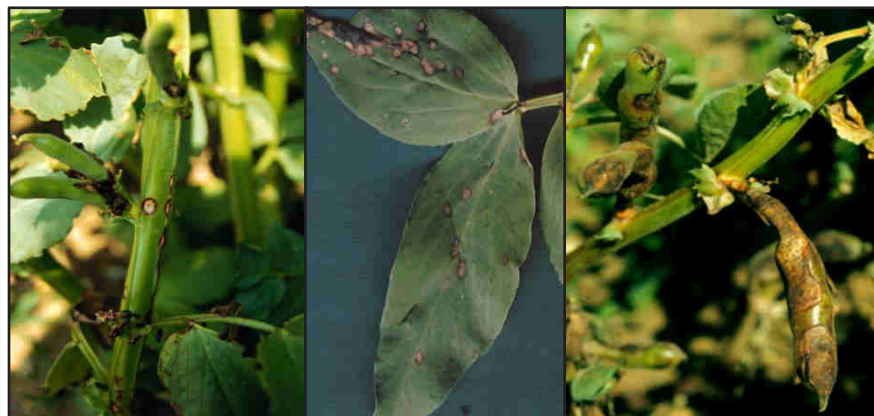
**Key words:** Ascochyta, biotechnology, breeding, crop management, legumes, resistance

Cool season food legumes are affected by a number of foliar and root diseases, being ascochyta blights one of the most important group of diseases worldwide. Ascochyta blights are incited by different pathogens in the various legumes, namely *Ascochyta rabiei* (Pass.) Labr. (teleomorph *Didymella rabiei* Kovatsch. ex Arx) in chickpea, *A. fabae* Speg. (teleomorph *D. fabae* G.J. Jellis & Punith.) in faba bean, *A. lentis* Vassiljevsky (teleomorph *D. lentis* W.J. Kaiser, B.C. Wang & J.D. Rogers) in lentil and *A. pisi* Lib., *Phoma medicaginis* var. *pinodella* (L.K. Jones) Boerema (previously *A. pinodella* L.K. Jones) and *A. pinodes* L.K. Jones (teleomorph *D. pinodes* (Berk. & A. Bloxam) Petr.) in pea (5, 7, 9) (Figs. 1-3).

A number of control strategies have been developed including cultural practices and chemical control but only marginal successes have been achieved. Historically, agronomic and cultural management options involved avoiding sowing close to infested legume stubbles and delaying sowing what is not always an option due to the short season or incurs in unsustainable yield penalties (4). Breeding for resistance is widely acknowledged as the most economic and environmentally friendly control method. However, resistance breeding is not an easy task.

Sources of partial resistance to ascochyta blight have been identified in all the cool season legumes, and are being used in breeding programmes. However, results are slow due to the limited availability of sources of resistance, the inaccuracy of screening methods and the quantitative expression of the resistances identified so far (5, 6, 9). Morphological and physiological characteristics of plant such as plant height, growth habit, canopy morphology, lodging and precocity can affect infection and development in the field due to their effect on microclimate in the canopy and conidia splash dispersal. Therefore, reduced infection observed under field conditions may result from either physiological resistance or avoidance, or their combination.

Many genetic studies have been performed in the various crops, listing a number of QTLs or even major genes providing incomplete resistance (reviewed in 4, 5, 8). Molecular markers linked to these genes/QTLs have been reported but practical application of MAS in cool season food legumes has been limited. Identification of new resistance sources of additional germplasm lines or wild relatives will make it possible to continue to improve on that resistance. The prospect of pyramiding of genes or QTLs, once identified, from various sources with the aid of modern molecular techniques remains a possible fruitful approach for further improving resistance to ascochyta blights in cool season food legumes. We are now also facing an accelerated progress in the genomic and biotechnological research, which will soon provide important understanding of some crucial developmental mechanisms in both the parasites and their host plants and will provide candidate genes for resistance (1, 2, 3). The application of next generation sequencing technologies will provide a new research framework and molecular tools to be applied in resistance to ascochyta blights in legumes. The ongoing analysis of *Ascochyta* genomes at various laboratories will also provide unique information on virulence genes to be used as target for breeding.



**Figure 1.** Typical *D. fabae* lesions in faba bean stems (left), leaves (middle), and pods (right)

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**Figure 2.** Chickpea cultivar susceptible to *D. rabiei* surrounded by resistant ones (left); detail of lesion on a pod (right)



**Figure 3.** Ascochyta blight in pea

These biotechnological advances will no doubt speed breeding success. However, insufficient understanding of the pathogen populations and imprecision of screening methods remain as major bottlenecks for successful ascochyta resistance breeding. There is a pressing need for a better understanding of pathogen variability, and for the standardization of screening procedures. Although a number of screening methods are available at detached leaf level or at seedling level in controlled conditions,

results insufficiently correlate with the disease reaction under field epidemic conditions. Greater accuracy in the measurement of resistance is needed also to reduce the confounding effects of varying phenology, canopy structure and plant density. This should be complemented with further understanding of epidemiology, determining the plant characteristics enhancing resistance and developing predictive information regarding use of fungicides. 

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# Molecular characterization of *er1* resistance to pea powdery mildew

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**Abstract:** The powdery mildew disease is a major threat for the cultivation of many plant species, including pea (*Pisum sativum* L.). Following an homology-based cloning approach, we recently characterized the powdery mildew resistance source *er1*, used worldwide in pea breeding programs, as the result of loss-of-function mutations in the *PsMLO1* gene. Owing to this study, a series of *er1* functional molecular markers, targeting polymorphisms causally determining the outcome of the pea/powdery mildew interaction, was developed. Taken together, these findings are expected to be of interest for future breeding activities aimed at introducing powdery mildew resistance, in pea as well as in other crops.

**Key words:** *er1*, pea, powdery mildew, resistance

It has been shown that recessive resistance to PM pathogens can be due to loss of function mutations in specific homologs of the *Mildew Locus O* (*MLO*) gene family, encoding for proteins with seven transmembrane domains. Specifically, this kind of resistance (known as *mlo* resistance), has been originally discovered in barley, and later shown to occur in Arabidopsis and tomato (4). Resistance is associated with the rapid formation of cell wall appositions (papillae) at plant-pathogen interaction sites, which are thought to prevent fungal penetration into epidermal cells.

## *er1* resistance is caused by loss-of-function polymorphisms in the *PsMLO1* gene

Analogously to *mlo* resistance, *er1* resistance is recessively inherited and acts at the pre-penetrative stage. Such genetic and phytopathological similarities prompted us to test whether *er1* resistance could be another case of *mlo*-based immunity. Genomic database search allowed the identification a pea *MLO* gene, *PsMLO1*, phylogenetically close to barley, Arabidopsis and tomato *MLO* homologs previously associated with resistance with the interaction with PM

## Introduction

The powdery mildew (PM) disease affects thousands of plant species, and is a major threat for pea (*Pisum sativum* L.) cultivation worldwide. Chemical control of pea PM is costly and raises environmental concerns; therefore, the use of resistant cultivars is highly desirable. So far, three PM resistant genes have been found in pea germplasm, two recessive (*er1* and *er2*) and one dominant (*Er3*). However, the *er1* gene is by far the most widely used resistance source in breeding programs (Fig. 1).



**Figure 1.** Phenotype of a pea *er1* genotype (left) and the commercial cultivar Sprinter (right) following artificial inoculation with the powdery mildew pathogen *Erysiphe pisi*

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**Table 1. Characteristics of the seven *er1* functional markers reported so far in scientific literature, discriminating *PsMLO1* loss-of-function mutants from wild type**

<i>er1</i> allele	Mutation in <i>PsMLO1</i> sequence <sup>a</sup>	Marker type	Forward and reverse primer sequences 5'-3'	PCR products (bp)	Restriction enzyme	Expected restriction fragments size (bp)
<i>er1-1</i>	C <sup>680</sup> →G	CAPS	AGGTTTGCAAGGGACACAAC TGAAGAAGCTAACCTGATTCAACC	195	<i>AsuHPI</i>	<i>er1-1</i> : 195 wild-type: 148, 47
<i>er1-2</i>	Unknown size insertion	STS	CCAAAGGAGGAAAGGAAAC GGAGCAGGTGACAGGAGAC	<i>er1-2</i> : ~2700 wild-type: -	-	-
<i>er1-3</i>	ΔG <sup>862</sup>	dCAPS	CAATTGAAGAGGATTTTAAAGTTGTTCTAG GCCAGATAGTTGGACTGCAAG	312	<i>XbaI</i>	<i>er1-3</i> : 286, 26 wild-type: 312
<i>er1-4</i>	ΔA <sup>91</sup>	CAPS	GCTGTTGCAGTTGTGTGCTT GAACAAGGATGCCAAGTTGA	518	<i>AgsI</i>	<i>er1-4</i> : 248, 220, 50 wild-type: 248, 220, 39, 11
<i>er1-5</i>	G <sup>570</sup> →A	HRM	GATGAGGAAGTGGAAGACTT AATTGATATTCAACTGTTCTTGTC	54	-	-
<i>er1-6</i>	C <sup>680</sup> →G	CAPS	GATCCTGAGAGGTTTAGGT CAACTCTACTGATAGATCCAAAG	240	<i>HphI</i>	<i>er1-6</i> : 240 wild type: 178, 62
		STS	AGCATGTGGGCTCCGTG CATCATGTCCTGGAGGAAG	<i>er1-6</i> : 341 wild-type: -	-	
<i>er1-7</i>	G <sup>939</sup> →A	CAPS	TATATGGGCAGGGTGGT AGGTGCACCCCTTGATTAC	225	<i>CviKI-1</i>	<i>er1-6</i> : 225 wild type: 31+194
		STS	ATCTTCCTCCAGGACATG AATTAGTGGAAGAAATGGTAGT	<i>er1-7</i> : 372 wild-type: -	-	

fungi. Sequencing of *PsMLO1* in a novel *er1*PM resistant line obtained by experimental mutagenesis revealed the occurrence of a G → A transition with respect to the wild-type, resulting in a severely truncated protein. The homozygous state for the mutation was found to be invariably associated with PM resistance in a F<sub>2</sub> segregant population, providing support for the identification of a functional polymorphism determining the outcome of the interaction (5). Final evidence that *er1* resistance is due to loss-of-function mutations of *PsMLO1* has been provided by the work of Humphry et al. (3), showing the restoration of PM susceptibility in a *er1* line

following *PsMLO1* transient expression in leaves. In total, sequencing of *er1* resistant germplasm allowed the identification of seven distinct *PsMLO1* mutations, all of them predicted to result in protein loss of function (3, 6, 7, Table 1).


### Functional markers to aid *er1* selection

The identification of molecular markers linked to the *er1* allele has been for a long time one of the main aims of pea breeding. Indeed, phenotypic selection of *er1* resistance is complicated by its recessive nature, which does not allow discriminating heterozygous individuals; moreover, it requires time consuming and costly disease tests and might be complicated by environmental variables.

Having characterized *er1* resistance as the result of *PsMLO1* loss-of-function, we addressed further research to the identification of functional markers, i.e. markers targeting *PsMLO1* polymorphisms causally associated with phenotypic trait variation. In total, we developed a panel of Cleaved Amplified Polymorphism (CAPS), Sequence Tagged Sites (STS) and High Resolution Melting (HRM) markers targeting all the five loss-of-function *PsMLO1* mutations at the time characterized in pea germplasm (5, 6). More recently, Santo et al. (7) reported the characterization of CAPS and STS markers aiding the selection of two novel *PsMLO1* loss-of-function alleles, obtained by experimental mutagenesis (Table 1).

## Conclusion and perspectives

The molecular basis of *er1* resistance, first described over 60 years ago, has been finally unravelled. *er1* resistance is still very effective in most pea agricultural settings, although there are recent reports of isolates of the PM species *Erysiphe pisi* DC., *E. trifolii* Grev. and *E. baeumleri* (Magnus) U. Braun & S. Takam. causing disease on *er1* genotypes (1, 2). Therefore, the development of a complete set of *PsMLO1*-based functional markers, overcoming limitations of previously reported *er1*-linked markers (such as recombination and poor transferability across genotypes), is predicted to be of great value for future breeding activities.

An important consequence of the molecular characterization of *er1* resistance is to substantiate the validity of a general breeding strategy based on the selective inactivation of *MLO* homologs, which can be conveniently exploited in order to introduce PM resistance across agricultural species, including legumes (4). To this respect, it is worth to mention the recent characterization of PM resistant phenotypes in pepper, following virus induced silencing of the *MLO* homolog *CaMLO2*, and bread wheat, following targeted mutagenesis of three *MLO* homeoalleles by using transcription activator-like effector nuclease (TALEN) technology (8, 9). 

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# Advances and prospects for development of multi-component microbial inoculant for legumes

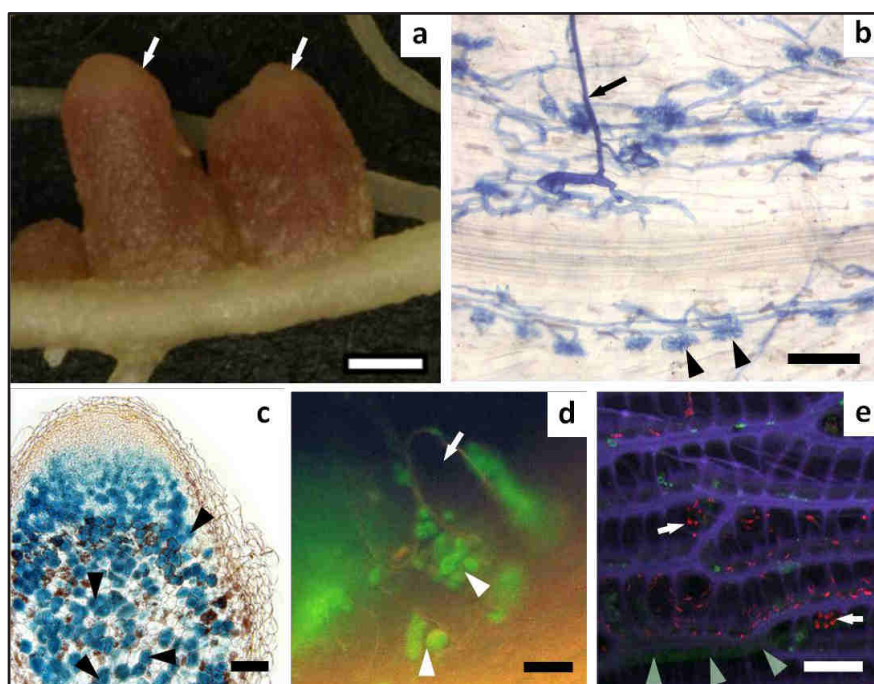
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**Abstract:** Nitrogen-fixing nodules formed by various proteobacteria on roots of the legumes (legume-rhizobial symbiosis, LRS), arbuscular mycorrhiza (AM) with glomeromycotan fungi, and associations with plant growth-promoting bacteria (PGPB) implement nutritional and defensive functions in plant, improve soil fertility, and thus are appropriate to be used for sustainable crop production and soil restoration. Here, we propose an approach to combine these symbioses in common multi-component plant-microbe systems with legume plants as a basis. Main progress, problems and prospects for development of multi-component microbial inoculants for legumes and non-legumes are discussed.

**Key words:** arbuscular mycorrhizal fungi, legumes, microbial inoculants, multi-component microbial fertilizer, plant growth-promoting bacteria, rhizobia

## Introduction

Legumes (*Fabaceae*) represent unique group of plants able to intimately interact with nodule bacteria collectively called rhizobia, with arbuscular mycorrhizal fungi (AMF), and with rhizospheric or/and endophytic plant growth-promoting bacteria (PGPB) (Fig. 1). These symbioses provide plant with nutrients (predominately nitrogen and phosphorus), protect it from biotic and abiotic stresses and improve soil structure and fertility (13, 33, 38, 39, 40). The vast majority of



**Figure 1.** Examples of agriculturally and ecologically important plant-microbe symbioses: (a, c) nitrogen-fixing nodules formed on the root of pea (*Pisum sativum*) inoculated with *Rhizobium leguminosarum* bv *viciae*; (a) general view, scale bar 1 mm; (c) light microscopy of longitudinal vibratome sections of the nodules colonized by rhizobia having  $\beta$ -glucuronidase reporter system - arrowheads point to bacteria differentiated into symbiotic forms, the bacteroids, scale bar 100  $\mu$ m; (b) arbuscular mycorrhiza of *P. sativum*, light microscopy of macerated roots colonized by fungus *Rhizophagus irregularis* (black ink staining) - arrow points to extraradical hypha, arrowheads to intracellular symbiotic structures, the arbuscules, scale bar 50  $\mu$ m; (d) fluorescence light microscopy of plant growth-promoting rhizobacteria *Pseudomonas chlororaphis* (carrying GFP reporter fusion) attached to the root surface of wheat (*Triticum monococcum*) - arrow points to a root hair, arrowheads, to bacterial microcolonies, scale bar 20  $\mu$ m; (e) endophytes of *Sphagnum fallax* moss gametophytes, confocal laser scanning microscopy image: *Eubacteria* (arrows) are visualized in hyaline cells of branch leaves by using fluorescent in situ hybridization with universal bacterial probes (EUB338, EUBII338, EUBIII338) - arrowheads point to hypha of an endophytic fungus (autofluorescence), scale bar 20  $\mu$ m

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commercial inoculants available at the moment are based on pure cultures of single microorganism, mainly due to the current procedures for governmental registration and certification of inoculants (10, 17, 20, 45). At that, the use of microbial inoculants

in legumes is dominated by an application of rhizobia to fix atmospheric nitrogen (43, 44). In this review, we propose to combine three aforementioned types of symbiosis in common multi-component plant-microbe system established around legume plant.



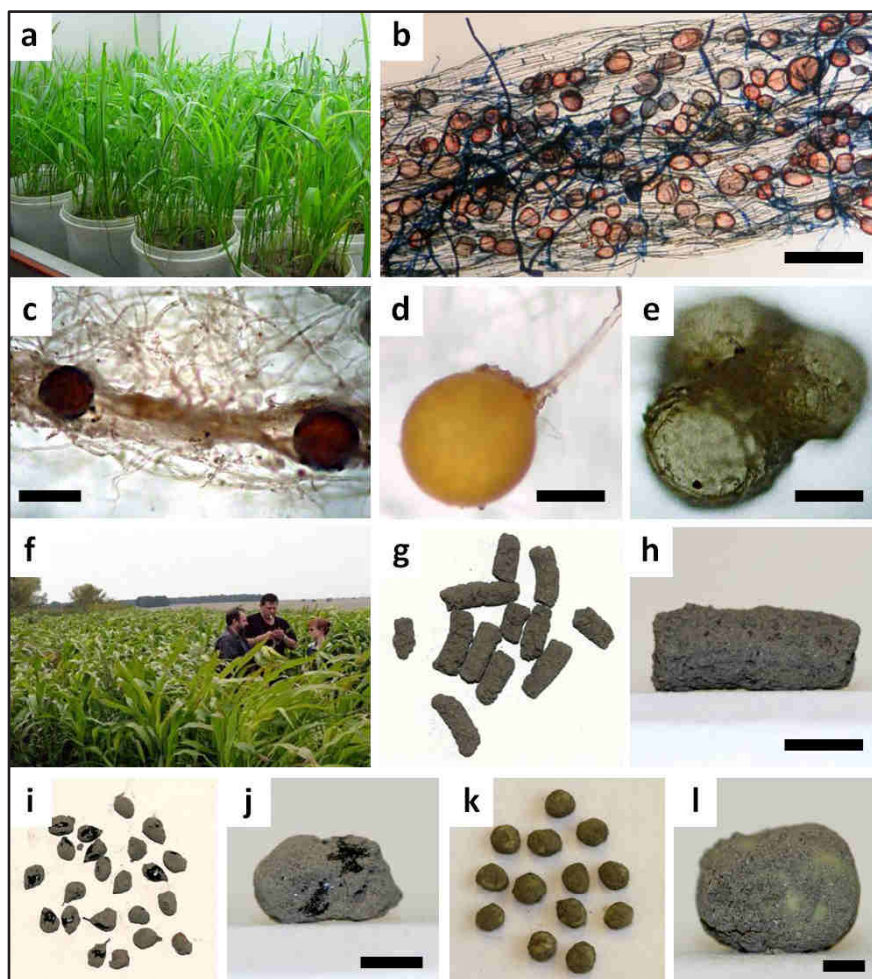
Main progress, problems and prospects for large-scale production of multi-component microbial inoculants for legumes (and non-legumes) are discussed.

### The large-scale production of bacterial inoculants

The large-scale production of rhizobia- and PGPB-based inoculants is not particularly difficult, since bacteria can be easily cultivated on various nutrient media with the use of fermenters. Once the particular strain for the inoculum has been selected, an industrial standardized process of production can be defined. The materials constituting the carrier can be of various origins: organic, inorganic, or synthesized from specific molecules. Availability and cost are the main factors affecting the choice of both growth media and carrier (23, 44, 45). There is increasing interest in clay- and peat-based granular inoculants applied to the soil, and in seed-applied liquid inoculants (13).

### Substrate-based technology of mass production of AMF inoculum

The mass production of AMF inoculum is more problematic, since AMF are not cultivated outside of plant (19, 40). The substrate-based inoculum is the cheapest one, and thus is much more frequently used for large-scale production. It is a mixture of mycorrhized roots of plants cultivated in containers or beds in greenhouse or in field with soil- or sand-based growth substrate containing AMF spores (Fig. 2a-f) (17, 19). One of the major disadvantages of this method is the necessity of large amounts of inoculum, which means the significant inputs for its transportation to the place of use. Thus, it seems to be expedient to utilize the local manufactures of inoculum (On-farm production) organized in the proximity to final consumers (14, 15). In addition, many inoculum producers of the Industry still develop formulations using different AMF, which quite often aren't properly characterized in terms of ecological, soil, host or pathogen requirements. The lack of awareness about the purity and effectiveness of AMF inocula leads to cross contaminations. These, and the lack of quality control for several marketed inocula, are amongst the main reasons for the low acceptance of mycorrhizal technology in agricultural practices (17).



**Figure 2. Substrate-based technologies of biofertilizers production on the basis of arbuscular mycorrhizal fungi (AMF) inoculums:** (a) production of an initial AMF inoculum for multi-component microbial fertilizer (MMF) in pots with *Sorghum* sp. grown in filtration-washing sediment as a substrate under inoculation with highly effective isolates of AMF; (b-e) propagules of AMF located in substrate-based inocula; (b) light microscopy of macerated *Allium schoenoprasum* L. roots colonized by *R. irregularis* (black ink staining) - arrows point to intercellular vesicles, scale bar 100  $\mu$ m; (c) light microscopy of intact *Zea mays* L. roots colonized by *Glomus* sp. - arrows point to intraradical spores, scale bar 50  $\mu$ m; (d, e) extraradical spore (d) and sporocarp (e) of *Glomus* sp., scale bar 50  $\mu$ m; (f, i, j) long-scale production of AMF inoculum for MMF; (f) *Sorghum* sp. grown on filtration field under inoculation with initial AMF inoculum (in the form of seed coating), scale bar 2 mm; (i, j) *Sorghum* sp. seeds coated with initial AMF inoculums; (g, h) MMF formulated into granules, scale bar 5 mm; (k, l) *P. sativum* seeds coated with MMF, scale bar 2 mm

### Mass production of AMF inoculants with the use of Root Organ Culture

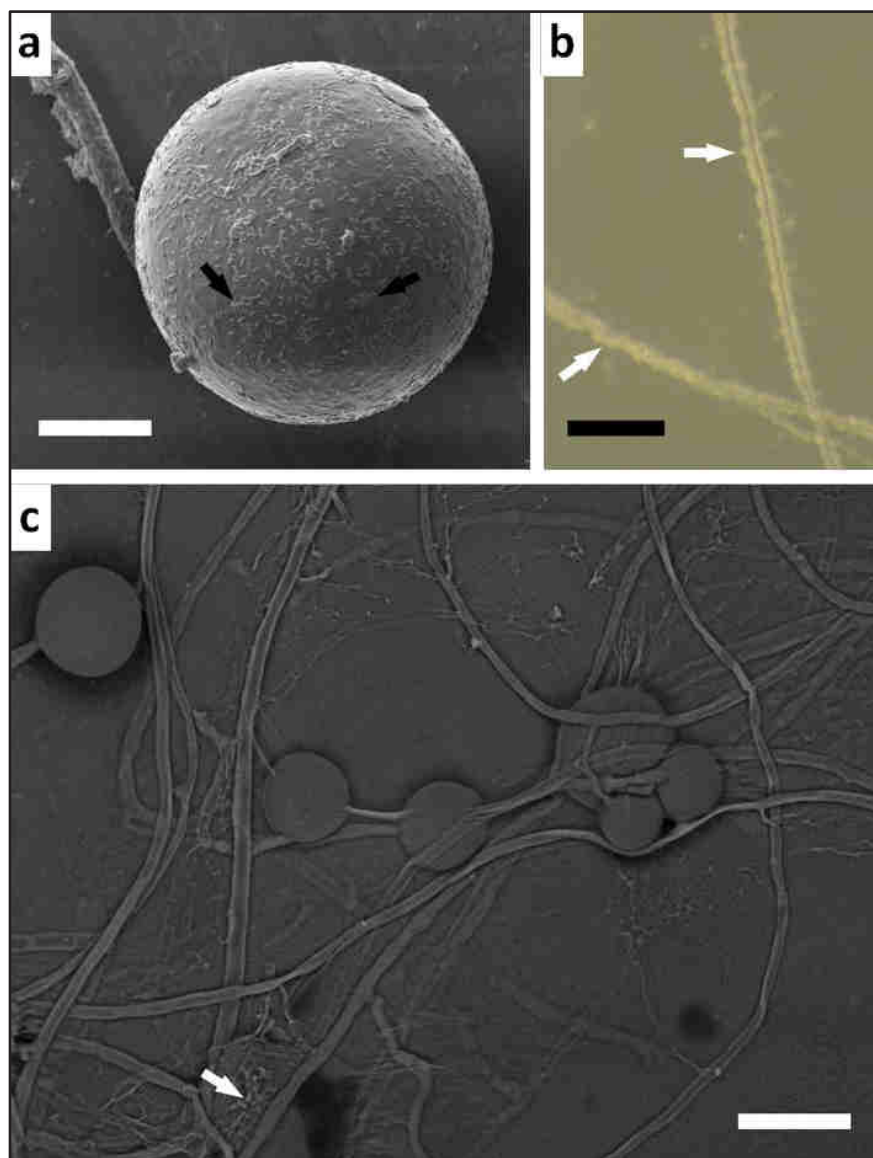
An alternative technology for producing AMF inoculum exploits the genetically modified host roots (Root Organ Culture, ROC) using the bacterium *Agrobacterium rhizogenes* carrying Ri T-DNA plasmid. This enables to produce viable, healthy, genetically pure and high quality fungal

propagules in large quantities without any pathogenic contamination under *in vitro* sterile environment (12, 28). The technology was transferred to many industries and resulted in the development of commercialized products which are ready available to end users for application. Mycorrhizal fungus inocula have been formulated as powdered seed coatings, tablets, and suspended in carrier for fluid drilling (12, See Chapter 17, Fig. 1-2; 14, 19).

### Background of the development of the multi-component microbial inoculants

The existence of cooperation between the rhizosphere microorganisms and their synergistic effect on plant growth (4, 5, 16), which is largely based on the evolutionary commonality of various plant-microbe symbioses (35, 36), allows us to develop a wide range of biotechnologies to increase both yield and quality of the crop production and reclaim desertified ecosystems with the use of multi-component legume-microbe systems. The possibility of using of multi-component microbial inoculants in sustainable legume crop production to reduce the doses of agrochemicals (6, 7, 34) and several advantages over mono-inoculation (6, 7, 21, 22, 24, 25, 29) was demonstrated. All this casts doubt on the appropriateness and the very possibility of applying mono-inoculants. Taking into consideration that naturally propagated fungi possess intracellular bacteria-like endosymbionts (5, 7) and bacterial satellites attached to the surface, one may even question the use of the term 'mono-inoculation' itself with respect to AMF inoculants produced with the substrate-based technique (19, 37).

Given that indigenous microbial communities are stable, the introduced microbes would more likely occupy only a very small niche in the whole community, or even get eliminated in a first week after introduction. The beneficial properties of the preliminary carefully selected strains introduced into natural ecosystems can rapidly be decreased or even lost because microbial genomes are remarkably unstable (3, 8, 27). Hence, during the development of the new biotechnologies, it is recommended to use a complex of local microbial isolates, adapted to particular environmental conditions (3, 15, 29, 30, 31, 42). Thus, one of the important problems of the modern agricultural microbiology is the creation of consortia of soil microorganisms isolated from natural environment, which are not only beneficial for plant, but also complement each other instead of being antagonistic.



**Figure 3. Co-culture of arbuscular mycorrhizal fungus (AMF) *Rhizophagus intraradices* and plant growth-promoting bacteria (PGPB) *Pseudomonas fluorescens* derived from root organ culture (ROC): (a) scanning electron microscopy (SEM) image of AMF spore with bacteria attached to its surface, scale bar 20  $\mu\text{m}$ ; (b) fluorescence light microscopy of AMF extraradical hyphae colonized by bacteria, scale bar 200  $\mu\text{m}$ ; (c) SEM image of *in vitro* co-culture of AMF and bacteria. (Scale bar: 100  $\mu\text{m}$ ) - arrows point to bacterial colonies**

### Substrate-based technology for the production and application of multi-component microbial fertilizer (MMF)

A rare example of a fully-developed technology for production and application of a multi-component microbial inoculant is the one based on an unsterile compost-like

substrate. The substrate results from the storage of filtration-washing sediment, a by-product of sugar production from sugar beet, on filtration fields during 20-30 years. Utilization of this type of waste is an important environmental problem. This substrate (pH about 7) consists of soil and lime and possesses its own microbial society including AMF, rhizobia and PGPB. All dominating bacterial strains demonstrate




high plant growth-promoting activity (1, 2, 9). When preparing the MMF, the substrate primarily is used for AMF-inoculum production on *Sorghum* sp. under inoculation with highly effective isolates of AMF (Fig. 2 a, f, i, j). The inoculum then is enriched with a mixture of active strains of rhizobia and PGPB and formulated into granules or seed coatings (Fig. 2 g, h, k, l) (9). The effectiveness of this approach as well as positive after-effects were demonstrated in year-by-year field trials with legumes and non-legumes (22, 25, 34, 46).

The bottleneck of the MMF, as a substrate-based inoculum, are the difficulties with its quality control, monitoring of the introduced microorganism in agricultural soils, and especially screening for the presence of pathogenic organisms in the carrier substrate. Solving of these problems is the task of near future. Molecular techniques could be used to trace the survival of AMF after introduction into the environment. DNA barcoding based on metagenomic approach (NGS) seems to be the most promising for the purpose and is being actively developed (11, 26, 41).

Despite aforementioned shortcomings, MMF-based approach best suits the demands for inoculum used for legume breeding to improve their effectiveness of interaction with beneficial soil microbes, since it would increase the content and biodiversity of microbes in agricultural soils (37).

## Prospects

In the future similar inocula could obviously be developed with ROC as a basis, since AMF could be successfully cultivated *in vitro* together with mycorrhiza-helper bacteria (MHB) (18, 32). TERI (New Delhi, India) has developed ROC-based technique specially to co-culture AMF and bacteria (Fig. 3). The microbes possessing both PGPB and MHB features could be isolated from natural environment along with rhizobia and then established in ROC of AMF. In order to develop effective AMF-bacterial consortia for creation of complex microbial inoculum as well as continuous co-culture, it is important to study how these combinations would help each other and how these microbes impact the rhizosphere biodiversity.

The ROC technology would be most appropriate to modern requirements for microbial inoculum for their governmental registration and certification. It will also make them more accessible and convenient for use by farmers in the manufacturing of agricultural products based on legume (as well as non-legume) crops. 

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## Second International Legume Football Cup

by FIFA\*


One fine September evening in 2009, after an exhausting meeting of the Organising Committee of the legume conference held in Antalya, Turkey, one grain legume breeder remarked to one forage legume breeder that he had seen a very nice football pitch within the hotel complex. An idea of organising what became the International Legume Football Cup (IFLC) had been definitely conceived (Fig. 1). On the margins of the merged 5th International Food Legume Research and 7th European Conference on Grain Legumes Conferences, the First International Legume Football Cup was held in late April 2010, with FC *Cicer*, FC *Lens*, FC *Pisum* and FC *Vicia* (1).

Following this rather young but most bright tradition, the Second International Football Cup (ILFC2) was held on 9th and 10th of May, 2013, as an integral and inevitable part of the First International Legume Society Conference, taking place in Novi Sad, Serbia. According to the society's character, four brand new teams were established: FC Forage Legumes, moved (or mowed) only forward by the tireless Beat Boller, FC Grain Legumes, schematically organised by the breeder's hand of Tom Warkentin, FC Intercropping, carefully modelled across the pitch by Laurent Bedoussac, and FC Soybean, with a masterful technique and technology of Aleksandar Ilić (Fig. 2).

Many were surprised to find out that IFLC also had its scientific and business sides: according to anonymous sources, certain players were offered to transfer from one team to another for up to ten published papers per year in the open access SCI journals with impact factors higher than 3.

Just like in Antalya, the results of the matches were either impossible to follow or completely unimportant. Having eliminated FC Forage Crops and FC Grain Legumes in the semi-finals, FC Intercropping and FC Soybean faced each other in the great final match. Perhaps it was the diet regime that was decisive in favour of FC Intercropping. Although reluctant to reveal their teams' private affairs, such as diets they were on, the journalists claim that FC Forage Legumes relied solely on fresh lucerne seedlings, FC Grain Legumes consumed a special pulse muesli and FC Soybean chose home-roasted early maturing seeds. On the other hand, FC Intercropping was nourished with legumes as building material, cereals as anti-hunger agent and rapeseed as  $\omega$ -3 biofuel: it may be assumed that this feeding design eventually provided them with a flattering title of the ILFC2 winner and the trophy-owner during the next three years (Fig. 3).

It is still early to say what will welcome us at ILFC3 during the Second International Legume Society Conference in Tróia, Portugal, in October 2016. There are proposals that the team names should be based upon specific legume research topics, such as, for instance, FC GWASgow Celtimorgans (for geneticists and breeders), FC Mealano (for nutritionists and pharmacists), FC Manstresser United (for abiotic and biotic stress experts) or FC Barcelonomy (for agronomists and agroecologists): more ideas are most welcome.

In the end, the renowned publishers Panini from Modena and Topps (ex-Merlin) from New York are already competing to be an official publisher the ILFC3 collectible sticker album... 

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Fédération Internationale de Fabaceaeuse Football Association



Figure 1. The official ILFC logo (above) and Leggy, the official ILFC mascot (below)



Figure 2. The official emblems of the ILFC2 teams

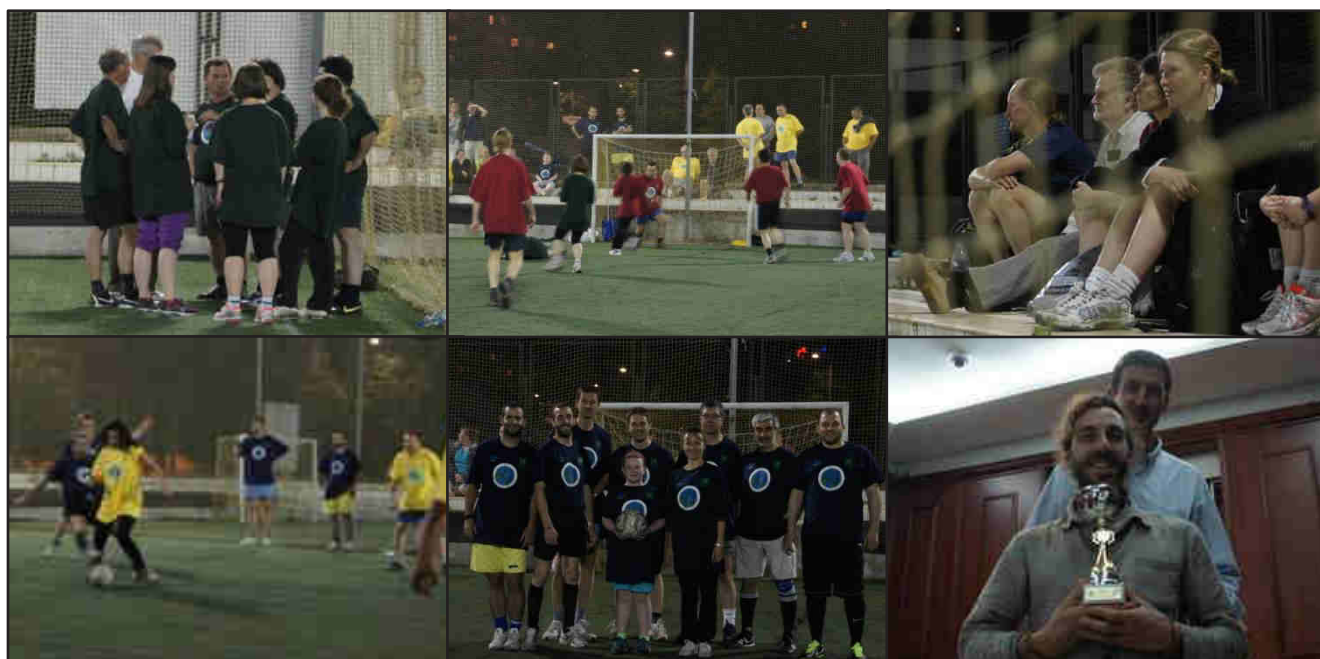


Figure 3. Selected moments of the Second International Legume Football Cup (ILFC2), Novi Sad, Serbia, May 2015: FC Forage Legumes (in green) develops the cutting schedule in its defense (above, left); FC Grain Legumes (in red) ready for a counter-dehulling (above, middle); the most faithful supporters in Antalya and Novi Sad (above right); FC Soybean performs Lord Tennyson's poem *The Charge of the Light (Yellow) Brigade* (below, left); FC Intercropping (in blue) in the stage of full champion flowering (below, middle); The ILFC2 trophy in the tendrils of a supporting crop accompanied by a supported crop (below, right)



# Legumes of the world of Narnia

by Aleksandar MIKIĆ

**Abstract:** This short essay was aimed at recording legume species in the world of Narnia, created by C. S. Lewis, as presented in the seven books of its chronicles. There are sparse references to a diverse range of legumes, comprising crops, shrubs and trees, demonstrating the importance they may have even in the fictional places and ages.

**Key words:** C. S. Lewis, legumes, Narnia

Clive Staples Lewis (1898-1963), a British writer, scholar, lay theologian, Professor at both Cambridge and Oxford Universities and many more, is perhaps best remembered worldwide as the author of *The Chronicles of Narnia*, a series of seven high fantasy novels published between 1950 and 1956, considered a timeless classic of children's literature. *Narnia* is the name of both a land and its magical world, created by the great lion Aslan in *The Magician's Nephew* (1955) and ended in *The Last Battle* (1956).

Despite numerous differences in style and other aspects, the *Narnia* novels have much in common, moral values and multi-layered narration, for instance, with those about another mythical world, *Arda*, created by Lewis' friend and colleague John Ronald Reuel Tolkien (1892-1973) and depicted in works such as *The Hobbit* (1936) and *The Lord of the Rings* (1954-1955).

Similarly to the flora of *Arda*, where several legumes are mentioned (1), the flora of the world of Narnia, with all its sharply contrasting regions, is often thoroughly and beautifully described. Interestingly, the legumes of Narnia appear in a variety of forms, including pulses and forages, as well as trees and shrubs (Fig. 1).

The lunch Lucy Pevensie had with the Magician Coriakin on his island in *The Voyage of the Dawn Treader* (1952), included green peas (*Pisum sativum* L.). Earlier in the same novel, she and her fellow travelers passed by Felimath, one of the Lone Islands, all covered with grass and clover (*Trifolium* spp.).

In *The Lion, the Witch and the Wardrobe* (1950), Edmund Pevensie, imprisoned by the White Witch, enjoyed the coming of spring, with laburnum (*Laburnum × watereri* (G. Kirchn.) Dippel) covered with gold. In *The Horse and His Boy* (1954), Bree and Shasta, on their long way northward, spend a night in a meadow with gorse (*Ulex* spp.) bushes.

If he had heard that soya bean (*Glycine max* (L.) Merr.) is added to the sausages in our own world (*The Silver Chair*, 1953), Trumpkin the dwarf would have said "It's all bilge and bean- (*Phaseolus* spp.) -stalks as far as I can see" (*Prince Caspian*, 1951).

*Diolch, Gyfaill.*

## Acknowledgements

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**Figure 1. A simplified map of the world of Narnia with the approximate locations of several recorded legume species in red-coloured initial letters: (C) clover, (G) gorse, (L) laburnum and (P) pea**

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## Second International Legume Society Conference (ILS2) *2016: Legumes for a Sustainable World*

Tróia, Portugal, 12-14 October 2016

The International Legume Society and the Instituto de Tecnologia Química e Biológica of the Universidade Nova de Lisboa cordially invite you to join us at the Second International Legume Society Conference, scheduled from 12-14 October, 2016 at Tróia resort, in the vicinity of Lisbon, Portugal.

In a world urgently requiring more sustainable agriculture, food security and healthier diets the demand for legume crops is on the rise. This growth is fostered by the increasing need for plant protein and for sound agricultural practices that are more adaptable and environmentally sensitive. Food, feed, fiber and even fuel are all products that come from legumes – plants that grow with low nitrogen inputs and in harsh environmental conditions. The Second Legume Society Conference will be held during 2016 - the United Nations' International Year of Pulses. The goals of this UN International Year include: the encouragement of connections throughout the food chain that would better utilize pulse based proteins; increase global production of pulses; better utilization of crop rotations; and to address challenges in the trade of pulses.

The conference will address the following themes: Legume Quality and Nutrition; Farming Systems/Agronomy; Abiotic and Biotic Stress Responses and Breeding; Legume Genetic Resources; and New "Omics" Resources for Legumes. The health and environment benefits, as well as, the marketing of legumes will be transversal topics throughout the conference. Special attention will be given to foster the interaction of researchers and research programs with different stakeholders including farmers and farmer associations, seed/feed and food industries, and consumers. For this, the conference will also be the site of the Final Meeting of the EU-FP7 ABSTRESS project, the Annual Meeting of EU-FP7 LEGATO project; and final dissemination events of EU-FP7-ERANets MEDILEG and REFORMA. The results and conclusions from these four important research programs will be shared with conference attendees.

Please join us in beautiful Tróia, Portugal from 12-14 October, 2016! Plan now to include the Second ILS Conference in your busy agenda. Kindly share this information with any colleagues dealing with legumes.

*Diego Rubiales, on behalf of the Scientific Committee*

*Pedro Fevereço, Carlota Vaz Patto and Susana Araújo, on behalf of the Organizing Committee*



**Local Organizers**

The Instituto de Tecnologia Química e Biológica / Universidade Nova de Lisboa (ITQB/UNL) will be responsible for organising the Conference, in cooperation with the International Legume Society. The official language of the Conference will be the English.

**Conveners**

Pedro Fevereiro - Universidade Nova de Lisboa (ITQB/UNL)  
 Carlota Vaz Patto - Universidade Nova de Lisboa (ITQB/UNL)  
 Susana Araújo - Universidade Nova de Lisboa (ITQB/UNL)

**Scientific Coordinator**

Diego Rubiales - CSIC, Córdoba, Spain

**Local Organizer Committee** (in alphabetic order)

Nuno Almeida - ITQB/UNL  
 Susana Araújo - ITQB/UNL  
 Ana Barradas - Fertiprado  
 Manuela Costa - Universidade do Minho  
 Isabel Duarte - Instituto Nacional de Investigação Agrária e Veterinária (INIAV)  
 Sofia Duque - ITQB/UNL  
 Pedro Fevereiro - ITQB/UNL  
 Susana Leitão - ITQB/UNL  
 Eduardo Rosa - Universidade de Trás-os-Montes e Alto Douro  
 Marta Vasconcellos - Escola Superior de Biotecnologia, Universidade Católica  
 Carlota Vaz Patto - ITQB/UNL  
 Manuela Veloso - INIAV

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 Shiv Kumar Agrawal - ICARDA, Syria  
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 Charles Brummer - University of California, USA  
 Adrian Charlton - FERA, UK  
 Gerard Duc - INRA, France  
 Noel Ellis - ICRISAT, India  
 Pedro Fevereiro - ITQB/UNL, Portugal  
 Judith Lichtenzweig - Curtin University, Australia  
 Kevin McPhee - North Dakota State University, USA  
 Aleksandar Mikić - Institute of Field and Vegetable Crops, Serbia  
 Eduardo Rosa - Universidade de Trás-os-Montes e Alto Douro, Portugal  
 Diego Rubiales - Institute for Sustainable Agriculture, CSIC, Spain  
 Fred Stoddard - University of Helsinki, Finland  
 Richard Thompson - INRA, France  
 Tom Warkentin - University of Saskatchewan, Canada



## Venue

The conference will be held in Tróia in the vicinity of Lisbon, Portugal. Tróia is a beautiful sand peninsula dividing the Sado River from the Atlantic Ocean.

The nearest airport is the Lisbon International Airport, about 50 Km away. Shuttles will be made available from and to Lisbon International Airport.

During the period of Roman occupation, date from the 1st century to the 6th century AD, Tróia was an island of Sado delta, called Ácala Island.

The Sado Estuary Nature Reserve, where dolphins swim, and the Serra da Arrábida Natural Park, where a full developed Mediterranean forest can be seen, are two of the main natural attractions nearby Tróia peninsula.

The Tróia Golf Championship Course is considered the best course in Portugal in the categories of difficulty and variety. It also stands in 20th place in the list of the best golf courses in Europe drawn up by the Golf World magazine.



## First tentative programme

### October 10th and 11th, 2016

Ascochyta Workshop  
Satellite projects meetings (to be defined)

### October 11th, 2016

Evening: ILS2 Conference Registration

### October 12th, 2016

08:00 Registration  
09:00-09:30 Welcome addresses

#### 09:30-10:30 Session 1, plenary: Legumes value chain: market requirements and economic impact

09:30-10:00 Key lecture 1  
10:00-10:30 Key lecture 2

#### 10:30-11:00 Coffee break

#### 11:00-12:00 Session 2, plenary: Legumes and environment

11:00-11:30 Key lecture 1  
11:30-12:00 Key Lecture 2

#### 12:00-13:00 Poster viewing

#### 13:00-14:30 Lunch

#### 14:30 – 16:00 Parallel sessions

##### Session 3, parallel: Session 3, parallel: Mechanisms of beneficial legume-microbe interactions

14:30-15:00 Key lecture  
15:00-15:15 Oral presentation 1  
15:15-15:30 Oral presentation 2  
15:30-15:45 Oral presentation 3  
15:45-16:00 Oral presentation 4

##### Session 4, parallel: Genetic resources

14:30-15:00 Key lecture  
15:00-15:15 Oral presentation 1:  
15:15-15:30 Oral presentation 2  
15:30-15:45 Oral presentation 3  
15:45-16:00 Oral presentation 4

#### 16:00-16:30 Coffee break

#### 16:30-17:30 Parallel sessions

##### Session 5, parallel: Legumes value chain: market requirements and economic impact (cont.)

16:30-16:45 Oral presentation 1  
16:45-17:00 Oral presentation 2  
17:00-17:15 Oral presentation 3  
17:15-17:30 General discussion on Legumes value chain

##### Session 6, parallel: Legumes and environment (cont.)

16:30-16:45 Oral presentation 1  
16:45-17:00 Oral presentation 2  
17:00-17:15 Oral presentation 3  
17:15-17:30 General discussion on Legumes and environment

#### 17:30-18:30 Poster session 1

Slots of 3 min flash presentations (+ 2 min questions) from 12 selected posters on the sessions of the day

#### 20:45 Third International Legume Football Cup: semi-finals



**October 13th, 2016****8:30-10:00 Session 7, plenary: Legumes in food and feed and other alternative uses**

08:30-09:00 Key lecture 1

09:00-09:30 Key lecture 2

09:30-10:00 Highlighted oral presentation

**10:00-10:30 Coffee break;****10:30-12:00 Session 8, plenary: Frontiers in legume genetics and genomics**

10:30-11:00 Key lecture

11:00-11:30 Highlighted oral presentation

11:30-12:00 Highlighted oral presentation

**12:00-13:00 Poster session 2**

Slots of 3 min flash presentations (+ 2 min questions) from 12 selected posters from the sessions of the day

**13:00-14:30 Lunch****14:30 – 16:00 Parallel sessions****Session 9 parallel: Legumes in food and feed and other alternative uses (cont.)**

14:30-14:45 Oral presentation 1

14:45-15:00 Oral presentation 2

15:00-15:15 Oral presentation 3

15:15-15:30 Oral presentation 4

15:30-15:45 Oral presentation 5

15:45-16:00 General discussion on Legumes in food and feed and other uses

**Session 10 parallel: Frontiers in legume genetics and genomics (cont.)**

14:30-14:45 Oral presentation 3

14:45-15:00 Oral presentation 4

15:00-15:15 Oral presentation 6

15:15-15:30 Oral presentation 7

15:30-15:45 Oral presentation 8

15:45-16:00 General discussion of genetics and genomics

**16:00-16:30 Coffee break;****16:30-18:00 Parallel sessions****Session 11, parallel: Frontiers in plant and crop physiology**

16:30-17:00 Key lecture

17:00-17:15 Oral presentation 1

17:15-17:30 Oral presentation 2

17:30-17:45 Oral presentation 3

**Session 12 parallel: Integrated pest and disease management**

16:30-17:00 Key lecture

17:00-17:15 Oral presentation 1

17:15-17:30 Oral presentation 2

17:30-17:45 Oral presentation 3

**17:45-19:00 ILS General Assembly**

20:45 Third International Legume Football Cup: finals

## October 14th, 2016

### 8:30-10:00 Session 13 plenary: Frontiers in legume breeding

08:30-09:00 Key lecture

09:00-09:30 Highlighted oral presentation

09:30-10:00 Highlighted oral presentation

### 10:00-10:30 Coffee break;

### 10:30-12:00 Session 14, plenary: Frontiers in legume agronomy

10:30-11:00 Key lecture

11:00-11:30 Highlighted oral presentation

11:30-12:00 Highlighted oral presentation

### 12:00-13:00 Poster session 3

Slots of 3 min flash presentations (+ 2 min questions) from 12 selected posters from the sessions of the day

### 13:00-14:30 Lunch

### 14:30 – 16:00 Parallel sessions

#### Session 15, parallel: Advances in legume breeding (cont.)

14:30-14:45 Oral presentation 1

14:45-15:00 Oral presentation 2

15:00-15:15 Oral presentation 3

15:15-15:30 Oral presentation 4

15:30-15:45 Oral presentation 5

15:45-16:00 General discussion on advances in legume breeding

#### Session 16 parallel: Advances in legume agronomy (cont.)

14:30-14:45 Oral presentation 1

14:45-15:00 Oral presentation 2

15:00-15:15 Oral presentation 3

15:15-15:30 Oral presentation 4

15:30-15:45 Oral presentation 5

15:45-16:00 General discussion on advances in legume agronomy

### 16:00-16:30 Coffee break;

### 16:30-18:00 Parallel sessions

#### Session 17, parallel: Seed technology, marketing and knowledge-transfer

16:30-17:00 Key lecture

17:00-17:15 Oral presentation 1

17:15-17:30 Oral presentation 2

17:30-17:45 Oral presentation 3

17:45-18:00 Oral presentation 4

#### Session 18 parallel: Resistance to biotic and abiotic stresses

16:30-17:00 Key lecture

17:00-17:15 Oral presentation 1

17:15-17:30 Oral presentation 2

17:30-17:45 Oral presentation 3

17:45-18:00 Oral presentation 4

### 18:00-19:00 Concluding session

Posters and oral presentations awards

ILS Honorary member's awards

### 20:00 Farewell Dinner



## **International Legume Society (ILS)**

is publicly announcing

### **A CALL FOR TENDERS TO HOST THIRD ILS CONFERENCE (ILS3) IN 2019**

All interested organisations  
are kindly invited to express their interest to  
Professor Diego Rubiales, the ILS President,  
at [diego.rubiales@ias.csic.es](mailto:diego.rubiales@ias.csic.es),  
at the earliest convenience.

The venue of ILS3 will be defined and announced  
by the ILS Executive Committee  
in December 2015.

## Global Year of Pulses - 2016

Global Pulse Confederation (CICILS-IPTIC)

CICILS – IPTIC, shortly to be renamed Global Pulse Confederation is head quartered in Dubai and licenced under the Dubai Government authority, Dubai Multi Commodity Centre (DMCC). CICILS is the not for profit peak body for the whole global pulses industry value chain. As the sole international confederation for the industry it enjoys membership from 18 national associations (federations) and over 600 private sector members in an industry worth over \$100 Billion at the retail level and over 60 million tonnes in pulse production and distribution in over 55 countries. The organisation represents the common good of all sectors of the global pulse industry value chain from growers and researchers, through input and logistics suppliers, traders, exporters and importers to government bodies, multilateral bodies, processors, canners and consumers. CICILS works for transparency and sustainability in all sectors and aspires to contribute in as many ways possible to global food security and improved health and nutrition. The CICILS Executive Board consists of up to 30 members from all over the world elected from the membership. Board positions are voluntary, non-profit and carry no remuneration.

### OUR VISION

To create an inclusive global pulse organization recognized for its integrity, professionalism and ability to work together across the entire pulse value chain to resolve issues and grow the industry.

### OUR MISSION

To lead the global pulse industry to major crop status by facilitating free and fair trade and increasing production and consumption of pulse crops worldwide.

### OUR GOALS

- To expand the permanent membership of CICILS to include the broadest base of organisations and companies involved both directly and indirectly in the global trade of pulses.
- To ensure a reliable, consistent and safe pulse value chain delivering pulses that meet the requirements of the industry's existing and future customers and consumers - and to encourage all industry sectors that impact on production, marketing and service delivery for Pulses to operate ethically and at world's best practice.
- To identify, select, fund and/or otherwise support approved research and development activity that leads to increased production and consumption of pulse crops to address the critical health, sustainability and food security issues around the world.
- To work towards harmonisation of the global pulse trade and removal of all barriers to trade for pulses world wide, and where possible develop new markets.
- To hold annual conventions of the highest calibre, that unite CICILS-IPTIC global membership in friendship, provide a focus for exchange of ideas and information, and a forum for discussion and amicable resolution of industry issues.
- To support national and regional member associations through active participation in local country activities by local CICILS members ("Ambassadors").



## Themes

CICILS and its IYOP partners have identified a series of thematic areas that will be the focus for activities during the International Year. These areas represent the key issues where new and increased efforts could help make a difference in promoting sustainable agriculture and livelihoods, as well as healthy diets, through increased production, trade and consumption of pulses.

We are working on more than 100 activities and projects related to 2016, four of them have already been launched in the areas of branding, school programs, recipes, and market access. Fifteen external partners have been recruited to work on the year, from major science centres, health institutes, academia to farm groups. Additionally, a total of 30 national committees have begun activities in every continent.

These activities will be built around four thematic areas:

### 1) Creating Awareness

IYOP 2016 is an opportunity to increase awareness and global demand for pulses. We aim to reach an audience of 20-40 million people worldwide using social media, websites and global media outreach.

### 2) Food & Nutrition Security & Innovation

IYOP has set the ambitious targets of helping initiate:

- 20 governments to commit to including pulses as part of their food security policies.
- 100 research projects substantiating the ability of pulses to combat nutrition and health issues.
- 100 research projects into functional and nutritional properties for food product advancement.

### 3) Market Access & Stability

IYOP is an excellent opportunity to open a dialogue on improving the regulatory framework in which trade occurs. We hope to reduce trade barrier costs that are borne by farmers, processors, traders and consumers while introducing greater efficiencies to enhance food security, reduce price volatility and enhance the return to growers.

### 4) Productivity & Environmental Sustainability

IYOP 2016 is a perfect chance to draw the focus of the scientific community. We hope to see the completion of a 10-year plan of action on pulse research by the end of 2016 and the genome sequencing of three pulse crops by 2018.

## National Committees

CICILS has convened a worldwide network of promotional teams to ensure wide-reaching and global coordination of activities on the 2016 International Year of Pulses. The National Groups are made up of experts with “great ideas” who plan and coordinate the most important activities of IYoP outreach, from the ground up. Their work is essential to the successful dissemination of the key thematic areas of the Year.

The Groups will meet via a conference call every two months. The purpose of the calls is to provide an update on activities, exchange ideas, identify gaps and coordinate a global approach on the key themes of the IYoP. As of February 2015, there were 30 countries on the National Promotions Group mailing list and additions to this list will follow over the course of 2015 and 2016.

### Join Us!

We know you all love pulses, which is why we want to give you 10 ideas on what you and/or company can do to help promote the 2016 International Year of Pulses.

1. Include a link to [iyop.net](http://iyop.net) in your website.
2. Spread the word! Have your communications team promote pulse stories in the media. Messages like: "What Are Pulses and Why Are They Important?" can help.
3. Donate your recipes to the global collection, and feature the recipes on your web site. Send your recipes to [IYOP@emergingag.com](mailto:IYOP@emergingag.com).
4. Donate your photos to our Photo Gallery.
5. Be social and talk about us! Follow us on Twitter and use the hashtag #IYOP2016.
6. Make use of your own connections to get more supporters. Do you know a local company who could be a sponsor? Perhaps you know someone in the Agricultural Department in your country? We are here to coach you and to provide you materials on how to get them on board.
7. Share your news. Send us your pulse related news to include in the News pages of [iyop.net](http://iyop.net).
8. Submit your event to [iyop.net](http://iyop.net) to include on our Event Calendar.
9. Translate materials on [iyop.net](http://iyop.net) into your national language.
10. And finally... to welcome the Year, have an Event on January 5th, 2016 and serve pulses!





**North American Pulse Improvement Association Biennial Meeting**

**Niagara Falls, Canada, 5-6 November 2015**

<http://www.eventbrite.com/e/napia-2015-biennial-meeting-tickets-5457734230>



**20<sup>th</sup> Eucarpia General Congress**

**Zurich, Switzerland, 29 August - 1 September 2016**

<http://eucarpia2016.org>



**26th General Meeting of the European Grassland Federation**

**Trondheim, Norway, 5-8 September 2016**

<http://www.egf2016.no>



**XIV Congress of the European Society for Agronomy**

**Edinburgh, UK, 5-9 September 2016**

<http://esa14.org.uk>



**10th World Soybean Research Conference**

**Savannah, USA, 10-16 September 2017**

<http://www.wsrc10.com>

*Legume Perspectives* is an international peer-reviewed journal aiming to interest and inform a worldwide multidisciplinary readership on the most diverse aspects of various research topics and use of all kinds of legume plants and crops.

The scope of *Legume Perspectives* comprises a vast number of disciplines, including biodiversity, plant evolution, crop history, genetics, genomics, breeding, human nutrition, animal feeding, non-food uses, health, agroecology, beneficial legume-microorganism interactions, agronomy, abiotic and biotic stresses, agroecology, sociology, scientometrics and networking.

The issues of *Legume Perspectives* are usually thematic and devoted to specific legume species or crop, research topic or some other issue. They are defined by the Editorial Board, led by the Editor-in-Chief with the help from Assistant Editors, who select and invite one or more Managing Editors for each issue. Having accepted the invitation, the Managing Editor agrees with the Editorial Board the details, such as the deadline for collecting the articles and a list of the tentative contributors, from whom he, according to his own and free choice, solicit the articles fitting into the defined theme of an issue. A possibility that every member of the global legume research community, with a preference of the International Legume Society members or established authorities in their field of interest, may apply to the Editorial Board to be a Managing Editor and suggest a theme for his issue is permanently open and can be done simply by contacting the Editor-in-Chief by e-mail, with a clearly presented idea, structure and authors of the potential issue.

Since one of the main missions of *Legume Perspectives* is to provide as wide global readership with the insight into the most recent and comprehensive achievements in legume science and use, the articles published in *Legume Perspectives* are usually concise, clear and up-to-date reviews on the topic solicited by the Managing Editor from each author. Managing Editor is solely responsible for collecting the articles from the authors, anonymous peer-review, communicating with the Technical Editor and providing the authors with the proofs of their manuscript prior to the publication.

Apart from review articles, *Legume Perspectives* is keen on publishing original research articles, especially if they present some preliminary results of an outstanding significance for legume research and before they are published in their full volume, as well as brief reports on already held and announcements about the forthcoming national and international events relating to legumes, descriptions of the projects on legumes, book reviews, short articles on legumes in popular culture or everyday life, fiction stories on legumes and obituaries. The authors of such contributions are advised to contact the Editor-in-Chief first, in order to present the draft of their idea first and receive a recommendation if it is appropriate.

Regardless of the article category, *Legume Perspectives* prefers a clear, simple and comprehensive writing style that would make its articles interesting and useful for both academic and amateur audience. Your article is expected to assist in the exchange of information among the experts in various fields of legume research.

*Legume Perspectives* welcomes either longer (900-1,100 words + up to 3 tables, figures or photos + up to 10 references) or shorter (400-500 words + 1 table, figure, photograph or drawing + up to 4 references) manuscripts. The Editor-in-Chief, depending on the opinion of the Managing Editor, may allow any variation in length or structure, from case to case.

The manuscripts for *Legume Perspectives* should be prepared in Microsoft Office Word, using Times New Roman font, 12 points size and single spacing. Please provide each manuscript with a 100-word abstract and 4-6 key words listed alphabetically. The references should follow the style of the published papers in this issue, be given in full and listed alphabetically. The tables may be incorporated in the manuscript, while figures, photographs or drawings should be submitted separately as jpg files with a resolution of at least 600 dpi. The authors whose native language is not English are strongly advised to have their manuscripts checked by a native English speaker prior to submission and be persistent in following only one of all the variants of English they themselves prefer.

Publishing articles in *Legume Perspectives* is free.



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