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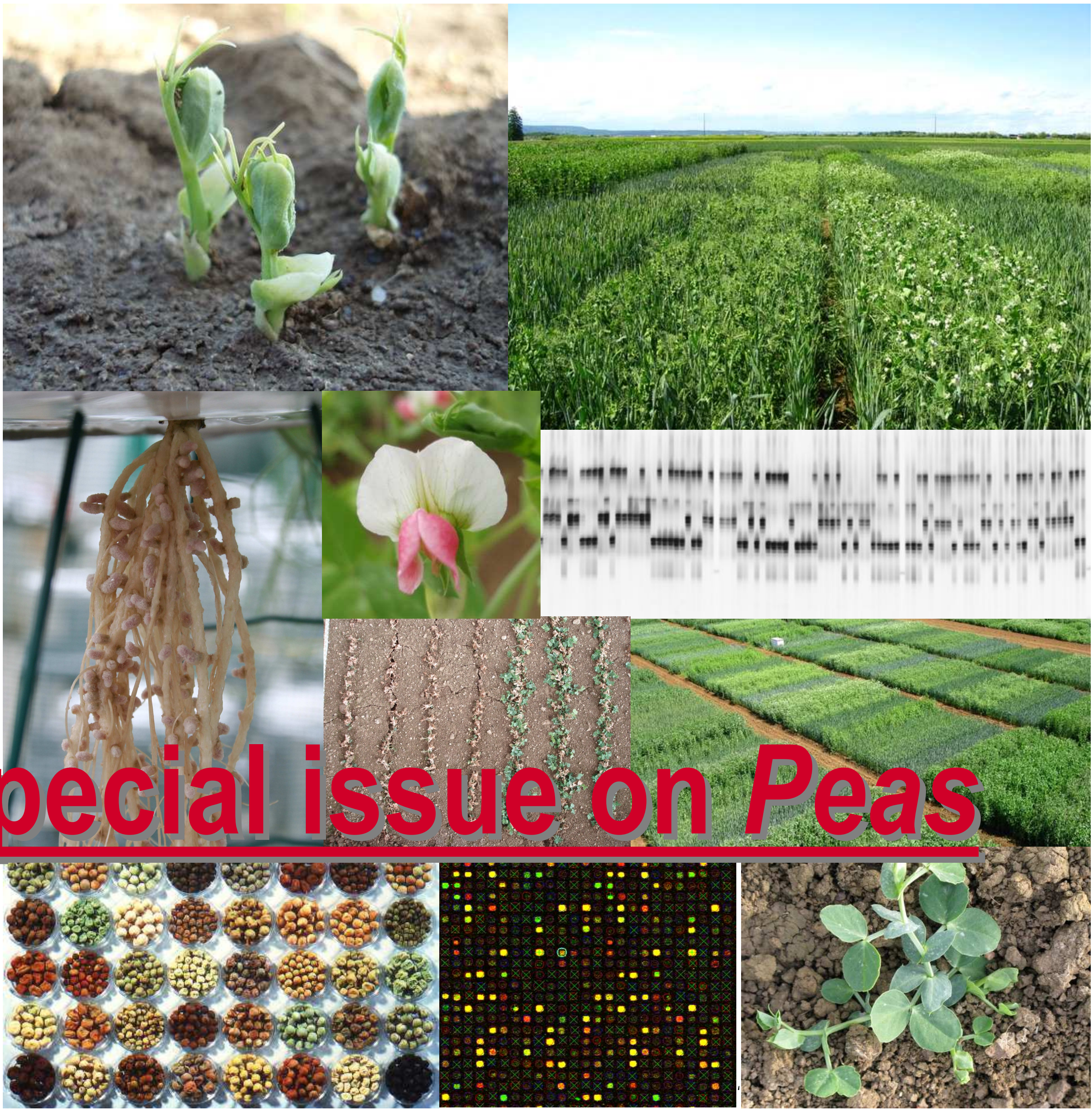

Special issue on *Peas*

EDITORIAL

I am proud to present this Grain Legumes issue dedicated to Peas. In spite of the recent difficulties encountered by the AEP community, the major editing activity of AEP has been pursued, thanks to the action of the members of the association. I would like to warmly thank and acknowledge people who contributed to it: Diego Rubiales, the president of AEP has launched this renewed series of Grain Legume magazines and entrusted me in the task to prepare this issue, A. Mikic made useful suggestions, N. Ellis and R. Thompson helped for papers' editing, and Dominique Millot was in charge of offset preparation, and last but not least, many thanks to the scientists who contributed by their reports to this issue. I hope you will enjoy the reading !

Judith BURSTIN

Managing Editor of GLM52

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New challenges and opportunities for pea

As long as 9-10,000 years ago, grain legumes played a central role in agriculture in the Fertile Crescent. Today, legumes still have a central role to play in sustainable agriculture. New challenges linked to food security and environmental preservation are emerging and these may raise public awareness of the need to introduce more grain legumes into agro-systems. In his speech on the 23th feb. 2009, M. Barnier, the French minister of agriculture declared that the production of plant proteins should be favoured in the next Common Agricultural Policy and a new French policy favourable to grain legume producers has been launched. New or re-discovered farming practices such as intercropping are being devised. Novel uses such as green chemistry products are arousing industrial interest in the pea crop.

Pea (*Pisum sativum* L.) is the most cultivated legume adapted to cool season areas in Europe. Pea is also and still an invaluable model plant in physiology and has been the source of major recent breakthroughs in the understanding of compound leaf development (Hofer et al. 2009), in deciphering the complex control of flowering (Liew et al. 2009, Wang et al. 2008) and in the discovery of a new plant hormone (Gomes-Roldan et al. 2008). During the last decade, more than 2000 recombinant inbred lines and a consensus linkage map of more than 500 markers (including SSR and SNP, Aubert et al. 2006) were developed. Researchers have identified genomic regions involved in the variability of numerous traits of interest (seed quality, frost and disease resistance). BAC libraries have been developed and large mutant populations are available such as the recently developed 5000 pea cv 'Cameor' TILLING mutant population (http://www.legumbase.fr).

But molecular tools are lacking to enhance marker-assisted selection and/or gene cloning in pea. By contrast, numerous genomic resources are available in the legume model species *M. truncatula* and the 3rd version of the sequence of the gene-rich regions of the *M. truncatula* genome has recently been released (http://medicago.org/genome/). This is a significant opportunity for pea, since all these resources can be used through translational genomics to enhance gene discovery in pea. Furthermore, new high-throughput sequencing technologies provide an opportunity to develop the tools that will enable to fill the gap between model species and cultivated species.

Aubert et al. 2006 Theor. Appl. Genet. 112: 1024-1041
Hofer et al. 2009 Plant Cell 21: 420-428
Liew et al. 2009 Plant Cell, 21 : 3198-3211
Gomes-Roldan et al. 2008 Nature 455: 189-U22
Wang et al. 2008 Proc Nat Acad Sci USA: 105: 10414-10419

A physiological study of weed competition in peas (*Pisum sativum* L.)

by Z. MUNAKAMWE

Peas dominate New Zealand grain legume production but weeds are a major problem. A research program to study weed control in peas was done through three field experiments over two growing seasons. Experiment 1 evaluated the effect of crop population on crop yield, and weed growth of Aragon, Midichi or Pro 7035 with and without cyanazine. Experiment 2 explored the physiology of two pea genotypes, leafed and semi leafless sown at three dates. A third experiment investigated the effect of different pea and weed population combinations on crop yield and weed growth.

A significant herbicide by population interaction in Experiment 1 showed that herbicide had no effect on seed yield at 100 and 400 plants m^{-2}. However, at 50 plants m^{-2} cyanazine treated plots produced 30% more seed yield than plots without herbicide. In Experiment 2 cyanazine treatment gave 19% more seed yield than unsprayed plots. A significant sowing date x genotype interaction showed that in the August sowing genotype had no effect on seed yield but in September Pro 7035 seed yield of 559 g m^{-2} was 40% more that of Midichi. In October it gave 87% more. Weed spectrum varied over time. In Experiment 3 pea seed yield increased with pea population. At 200 plants m^{-2} peas gave the highest seed yield (409 g m^{-2}) and at 50 plants m^{-2} the lowest (197 g m^{-2}).

It can be concluded that fully leafed and semi-leafless peas sown at similar populations give similar yields under weed free conditions. An increased pea sowing rate can increase yield particularly in weedy environments. Early sowing can also possibly control problem weeds in peas. Herbicide can enhance pea yield but can be replaced by effective cultural methods such as early sowing, appropriate pea genotype selection and high sowing rates.

Abstract of a thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy at Lincoln University, Canterbury, New Zealand. Email : George.Hill@lincoln.ac.nz

Dissection of the pea seed protein composition: phenotypic plasticity and genetic determinism

by M. BOURGEOIS

Europe has to face important and increasing needs for plant proteins, especially for animal nutrition. The pea crop, a grain legume for which seeds are rich in proteins (ca. 24% of dry matter), could fulfil these expectations. Nevertheless, seed protein content is still insufficient and unstable. Moreover, seed digestibility, closely linked to protein composition, needs to be improved to increase seed nutritional quality. In this context, the objective of this thesis was to provide a fine dissection of pea seed protein composition, to study its phenotypic plasticity and to elucidate its genetic control.

By a quantitative proteomics approach, we have produced a 2-DE reference map of the pea seed proteome (156 proteins were identified, among which a major part were storage proteins). The proteome exhibited a high complexity, probably resulting from the expression of multiple genes, as well as storage protein processing during seed development. Over three genotypes and three years of cultivation, the seed proteome displayed an elevated phenotypic plasticity, but the genotype effect was predominant. After having produced a genetic map focusing on functional markers involved in seed quality traits (535 markers in total), we have dissected the genetic determinism of protein composition. PQL (*Protein Quantity Loci*) of major seed proteins were detected. This genetic determinism appeared complex: 312 PQL were found for 191 spots. Common regulation mechanisms were shown, and most of the PQL had moderate effects, explaining less than 20% of the variance. These results have allowed us to identify key-regulatory regions underlying the modulation of protein composition. They may constitute interesting targets for the improvement of the nutritional quality of pea seeds. Furthermore, we have proposed candidate genes controlling seed protein composition.

PhD thesis, December 2008, University of Burgundy, Dijon.

Hormone discovery using the model plant *Pisum sativum* L

by C. RAMEAU[1]

The degree of shoot branching is an important component of plant architecture and has a major impact on plant fitness and on yield of most crops. During plant development, axillary buds, located at the axils of most leaves either will remain dormant or will outgrow to give a new lateral. This outgrowth is tightly regulated by environmental factors and endogenous signals. For several decades, it was established that auxin and cytokinin were the two major hormones controlling axillary bud outgrowth. Recently, a novel class of plant hormones, strigolactones, has been identified which acts as a branching inhibitor. From the first studies on apical dominance to the recent discovery of strigolactones, legumes and in particular pea, have contributed largely to these discoveries (1). In this paper, we present some of the key experiments that have been performed for branching studies and describe the main advantages of legumes for such experiments. In a next special issue of Plant Physiol on Legume biology, this presentation will be given in more detail (2).

Decapitation experiments : the classical theory of apical dominance

Several decades ago, the term apical dominance was defined as the inhibitory effect of the shoot apex on the outgrowth of the axillary buds located below. Pea was one of the first system used to study apical dominance. During the vegetative phase, most pea cultivars present dormant axillary buds at most nodes and axillary buds separated by long internodes make such studies easy. After decapitation, axillary buds that were dormant enlarge and some start to grow. The application of auxin to the stump of decapitated plants inhibits outgrowth of buds. The classical theory of apical dominance was that auxin produced in the shoot tip and moving basipetally down the stem inhibits axillary bud outgrowth.

In the 1930s, experiments on shoot branching with “two-shoot” plants led Snow to suggest that auxin was acting indirectly via a second messenger moving upward. These “two-shoot” plants were obtained by decapitation of young pea or *Vicia faba* seedlings to get two similar cotyledonary shoots. When only one cotyledonary shoot was decapitated, the axillary buds on this shoot were inhibited by the apex of the second intact shoot.

Next to auxin, cytokinin was the other plant hormone to have a strong influence on apical dominance as exogenous application of cytokinin directly to dormant axillary buds is able to stimulate their outgrowth. A decade ago, genetic evidence of a novel long-distance signal, other than auxin and cytokinin came from grafting studies between the high branching *rms* pea mutants and wild-type (WT).

Grafting experiments with high branching pea mutants: existence of a novel branching inhibitor

A mutant-based approach for the study of branching has been carried out in several species and mutations in the pea *RAMOSUS* (*RMS*) loci, the *MAX* loci in Arabidopsis, the *DAD* loci in Petunia and the *D* loci in rice give a high branching phenotype with relatively few pleiotropic effects. Grafting studies permit to locate sites of gene action and to demonstrate the role of long distance signals. In pea, epicotyl wedge graft using 7 d old plants is very easy to perform (Fig. 1). When a *rms1* mutant shoot is grafted on a WT rootstock, the branching is inhibited while this branching is not inhibited when a *rms4* shoot is grafted on a WT rootstock. These studies combined with hormone quantifications led to the model where *RMS1* and *RMS5* were acting both in rootstock and scion to control the level of a novel graft-transmissible branching inhibitor. *RMS3* and *RMS4* were mainly acting in the shoot to control the response to the branching inhibitor.

Figure1 : a 7 d old graft in pea

The cloning of these genes from the model plant Arabidopsis gave support to this model. *RMS1* and *RMS5* encoded Carotenoid Cleavage Dioxygenases (CCD8 and CCD7 respectively) which suggested that the branching inhibitor was carotenoid derived as the well known hormone abscisic acid. The response gene *RMS4* encodes an F-box protein which is consistent with RMS4 being involved in the transduction signaling pathway as TIR1 and COI1, receptors of auxin and jasmonic acid, respectively, encode F-box proteins which are typically involved in protein degradation via the ubiquitin-proteasome pathway.

Strigolactones (SL) as the novel branching inhibitor

The information that the branching inhibitor was carotenoid derived was an important clue to its discovery. In 2005, the group of H. Bouwmeester in Wageningen working on parasitic plants demonstrated that SLs were carotenoid derived. These molecules are produced by the roots of plants and exuded in the rhizosphere where they play major roles in both parasitic and symbiotic interactions. They promote arbuscular mycorrhizae (AM) symbiosis between more than 80% of plants and fungus. They also stimulate seed germination of parasitic plants belonging to the genera Striga and Orobanche which severely reduce the yields of economically important crops. To identify the genes involved in SL biosynthesis, researchers working on root colonization by AM fungi looked for mutants in Carotenoid Cleavage Dioxygenase (CCD) genes (they are 9 CCD genes in Arabidopsis, 6 being involved in ABA biosynthesis). One of the first important results obtained with the *rms* pea mutants was that root exudates of *rms1* failed to stimulate proliferation of fungus hyphae or Orobanche seed gmination when compared to WT exudates. Another essential result was the use of GR24, a synthetic analog of SL. The direct application of GR24 on axillary buds of

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rms1 mutant inhibited their outgrowth (at concentrations as low as 10 nM) while no inhibition was observed on axillary buds of the *rms4* response mutant. Here the facts that a series of well defined pea branching mutants were available, that pea is a mycotrophic plant (in contrast to Arabidopsis) and that axillary buds are easily accessible to exogenous treatments and to precise observations contributed to this discovery. At the same time another group from Japan used a series of high tillering rice mutants to demonstrate the implication of SLs i shoot branching (3).

A new field of research

SLs belong to the terpenoid family and present a common tetracyclic skeleton (Fig.2). Many enzymatic steps from the cleavage of a carotenoid precursor (still unknown) by CCD to SLs are still to be identified to have a complete under-standing of the biosynthesis pathway. Recent findings in rice have added one enzyme, D27 to the 2 CCD and the cytochrome P450 (MAX1) already known in the biosynthesis pathway. Identification of SL insensitive branching mutants will be essential for the dissection of the signaling pathways. The recent cloning of D14 in rice has confirmed that the SL signaling pathway will have some similarities with the signaling pathways characterized for other hormones. Future studies will have to answer to several questions such as how SLs interact with auxin and cytokinins to regulate branching or how environmental factors regulate branching. The role of SL in the induction of AM symbiosis is of particular interest. It has been shown that SL biosynthesis in roots is induced by low phosphorous and/or low nitrogen (which may induce the proliferation of AM fungi that will help the plant for its mineral nutrition). One major question is to know if this regulation of SL biosynthesis in roots has an impact on shoot branching. The discovery of SL as a new hormone should help to find new solutions to fight Orobanche and Striga which are two major pests in different parts of the world (and in particular for legumes).■

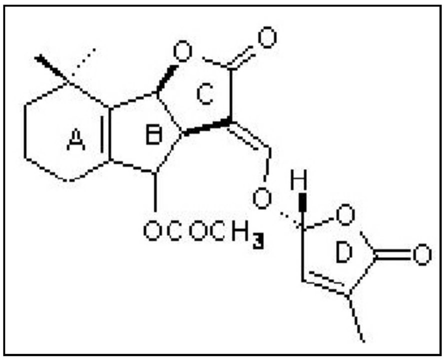

Figure.2. Structure of orobanchyl acetate, one of the major strigolactones found in Fabaceae

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(1) Gomez-Roldan, V. *et al*. Nature, 2008. 455 (7210): p. 189-94.

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Genetics of winterhardiness in pea

by I.LEJEUNE-HENAULT[1] and B. DELBREIL[2]

Need for winter peas and dissection of the winterhardiness character

Pisum sativum L. is the main european crop commonly used as a protein source in animal feeding. An important aim for (north) European pea breeders is the increase of yield and the extension of the production areas. Both these aims could be achieved by the use of winter pea varieties. Autumn sowings allow extension of the growth period between germination and flowering, resulting in naturally higher biomasses and seed numbers (5). But, when peas are sown in autumn, they develop in sub-optimal temperature conditions and, to survive winter, they have to withstand periods of frost that can vary in length and intensity, depending on the region. The dissection of the genetic determinism of pea winterhardiness would facilitate breeding of winter pea varieties. As regards winterhardiness in plants, three basic adaptation mechanisms can be distinguished: vernalization response, photoperiod sensitivity and cold acclimation. In the case of pea, the two last mechanisms are involved in winterhardiness and studied in our team.

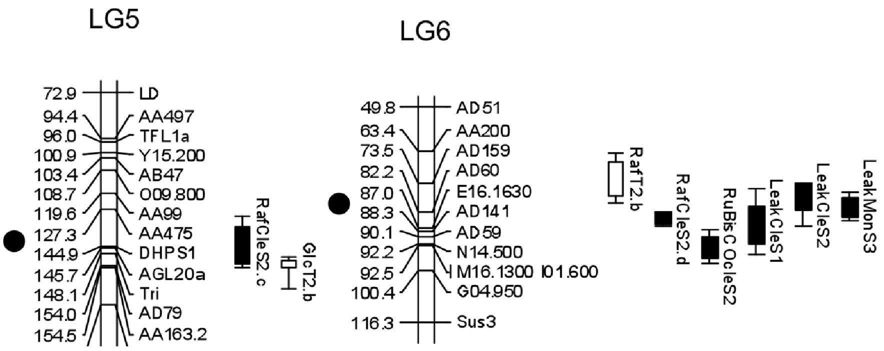

Figure 1, adapted from Dumont *et al.*, 2009 (2) Metabolites, physiological and morphological QTL on LG5 and LG6. Field data QTL (*black boxes*) in Clermont-Ferrand or Mons are identified by Cle or Mon, respectively. Controlled chamber QTL are represented with *white boxes*. *T2* sampling after 10 days of cold acclimation, *S1,S2* sampling during cold acclimation in the field, *Leak* electrolyte leakage, *Raf* concentration of raffinose, *Glc* concentration of glucose, *RuBisCO* activity of RuBisCO. *Black circles*, on the *left*, indicate the position of winter frost damage QTL detected within the whole population of recombinant inbred lines from field collected data.(With kind permission of Springer Science+Business Media).

Photoperiod sensitivity

Given that frost sensitivity rises after floral initiation and that the flowering period is also a key developmental stage for yield elaboration, the ideal winter pea could be defined as follows : it should initiate its flower primordia late enough to escape winter frosts and should flower early enough to escape drought and heat stresses in late spring (7). Consequently, we have paid a particular attention to flowering genes in pea, taking advantage of numerous studies describing the physiological and phenological effects of the main loci governing the transition to flowering in this species (6, for review). Among these flowering loci, *LF* (Late Flowering) and *HR* (High Response to the photoperiod) appear to be promising targets, as they are known to control respectively the intrinsic earliness for the beginning of flowering and the strength of the response to the photoperiod. More precisely, the dominant allele *HR* is known to delay floral initiation of autumn-sown peas until a longer daylength is reached in the following spring, this period also corresponding to more favourable temperature conditions. A quantitative genetics approach has been carried out to check the genetic linkage between the *HR* locus and frost tolerance in pea. A multi-locayion field evaluation of frost tolerance was undertaken in a population of recombinant inbred lines derived from a cross between the frost tolerant line, ‘Champagne’, and the frost sensitive line ‘Terese’. Champagne is also known to carry the dominant allele *HR*, conferring a delayed floral initiation under short days, comparatively to ‘Terese’ which carries the recessive allele. The colocalization of *HR* with the major forst tolerance QTL (Quantitative Trait Loci) identified within this population allowed us to confirm that this locus plays a prominent part in the control of frost tolerance in pea (3). We have now undertaken a marker-assisted breeding project aiming at associating the dominant allele at the *HR* locus with the earliest alleles known at the *LF* locus.

Cold acclimation

Most plants from temperate regions are able to increase their freezing tolerance in response to low but non-freezing temperatures, a process known as cold acclimation. Physiological features consistently associated with cold acclimation have been reported in many species among which a general up-regulation of primary metabolism, solute accumulation, changes in membrane physical properties and activation of the protection against oxidative stress (4).

We studied the cold acclimation process in pea using the same plant material as for the genetic study of photoperiod sensitivity, *i.e.* the parental lines ‘Champagne’ and ‘Terese’ and their recombinant inbred offsprings. Plants were grown in a climatic chamber in conditions that prevent floral initiation. At first, plants were exposed to moderately low temperature to induce cold acclimation, then they were submitted to freezing temperature in order to see the impact of acclimation on frost tolerance, and finally plants were transferred at positive temperature for a recovery period (Bourion *et al.* (1). Plants were sampled regularly during the different periods and their proteomic as well as

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physiological and biochemical traits were assayed. The response to cold acclimation was different between the two parental lines: ‘Champagne’ became tolerant to frost after cold acclimation whereas ‘Terese’ remained sensitive.

The genetic study of cold acclimation relied on complementary experiments in the field and in controlled conditions. We established that aside the *HR* QTL stated above (3), two other QTL for frost tolerance were consistently mapped on linkage groups LGV and LGVI both for frost damage recorded in the field and in controlled conditions. Several key parameters potentially involved in cold acclimation such as electrolyte leakage, concentration of sugars, and activity of RuBisCO were assessed in our mapping population. Some physiological QTL detected based on these mesasurements co-located with QTL of winter frost damages. In particular three raffinose QTL on LGV and LGVI were observed, one QTL of glucose concentration on LGV and one QTL of RuBisCO activity on LGVI (Figure 1). In addition, protein quantitative loci (PQL) were also mapped (data not shown). Amongst the 22 PQL co-locating with a QTL of winter frost damages on LGV, four could be attributed to carbohydrate metabolism and co-located with QTL of glucose and raffinose concentrations. Altogether, these results suggested that carbohydrate metabolism might play a major role in cold acclimation of pea, which is consistent with results already reported in the literature for other species. Quantitative trait loci for electrolyte leakage measured in field experiments were detected on LGVI. Electrolyte leakage is the physical measurement of membrane damage, which could be used to screen more quantitatively and objectively for tolerance to frost damages.

Need for *Medicago truncatula*

The density of the current genetic map of pea (one marker every 5.7 cM corresponding to 16,820 kb: 1 cM = 2,951 kb) and the relatively low number of identified genes within the QTL confidence intervals led us to carry out a transcriptomic approach in order to reveal new candidate genes that are progressively added to the initial map. To complete this strategy, we are starting a combined candidate gene/positional approach that relies on the synteny between pea and *Medicago truncatula*. For the three most determining winter frost damages QTL, we are going to use the first markers already mapped within the QTL confidence intervals in pea to identify *Medicago truncatula* BAC clones that will in turn provide new markers chosen in the literature for their role in cold acclimation and frost tolerance. This should allow us to identify more rapidly candidate genes underlying these QTL.■

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Combining plant genetic, ecophysiological and microbiological approaches to enhance nitrogen uptake in legumes

by V. BOURION[1]*, G. LAGUERRE[3], A.S. VOISIN[1], G. DEPRET[2], G. DUC[1] and C. SALON[1]

Legumes have a major environmental role to play in sustainable agriculture. First of all, the occurrence of symbiotic fixation of atmospheric N_2 reduces inputs of N fertilizer, and thus decreases the consumption of fossil energy and the risk of N-losses in the environment by NO_3-leaching and N_2O-emission. Moreover, their use in intercropping or by diversifying crop rotation may allow a lower input of pesticides (1). However, legume seed yields and protein contents are very variable, mostly because of their high sensitivity to the frequent environmental stresses. This review describes combined approaches for improving N nutrition in pea (2-5), which will help to define new varieties and cropping systems for sustainable agriculture.

Improvement of legumes N nutrition requires combined approaches of microbiology and plant genetics

Symbiotic nitrogen fixation (SNF) is performed by rhizobia inside the nodules, which are the symbiotic organs formed on the roots of the host legume. Thus, variability of nodulation must be explored considering both plant and microbial genetics, and the consequences of their interaction on N uptake performance. *Rhizobium leguminosarum* biovar *viciae* (*Rlv*) is the specific nitrogen-fixing symbiont of the legumes of the tribe Vicieae including pea. The genetic variability among the *Rlv* strains, especially for their *nod* gene type, was assessed to find the most performing strains for their symbiotic association with pea (3, 4). The host plant controls nodule number through a systemic negative feedback mechanism called autoregulation of nodulation (AON), and at least 3 genes in pea have already been shown to be involved in it: *NOD3* (6), *SYM28* and *SYM29* (7). Due to defective AON, mutations in all these genes induced a hypernodulated phenotype.

Creating hypernodulating genotypes was suspected to enhance SNF through an increase and prolonged nodule development and activity.

Nitrogen (N) nutrition of legumes also partly relies on soil mineral N absorption by roots. Thus, another strategy to improve exogenous nitrogen acquisition could be selection of lines with increased root system size. Despite the vital role of roots for both water and nutrient uptake and tolerance to root diseases, few experiments have investigated the genetic variation in root establishment of pea. Therefore, the N uptake performances of hypernodulating mutants, wild type and genotypes with high root development were compared in a field experiment (2).

Genetic variability for root and nodule development is associated with differences in N uptake

Differences in root biomass were observed among five genotypes studied in the field (2), with at beginning of seed filling (BSF) up to two-fold higher values for cv. Austin than for cv. Frisson (Figure 1). Highly significant differences in nodule biomass were also observed among genotypes, as early as at the 10-leaf stage until the BSF. The proportion of nodule biomass relative to total nodulated root biomass was highly variable, ranging from 5% for cvs Athos and Austin up to 13-20% for the hypernodulating mutants of cv. Frisson, P118 and P121 (*sym29* and *nod3* mutation, respectively). The difference in root and nodule repartition observed between the two mutants might reflect differences in regulation of nodulation between the *SYM29* and *NOD3* genes. Indeed, *SYM29* encodes for a receptor kinase

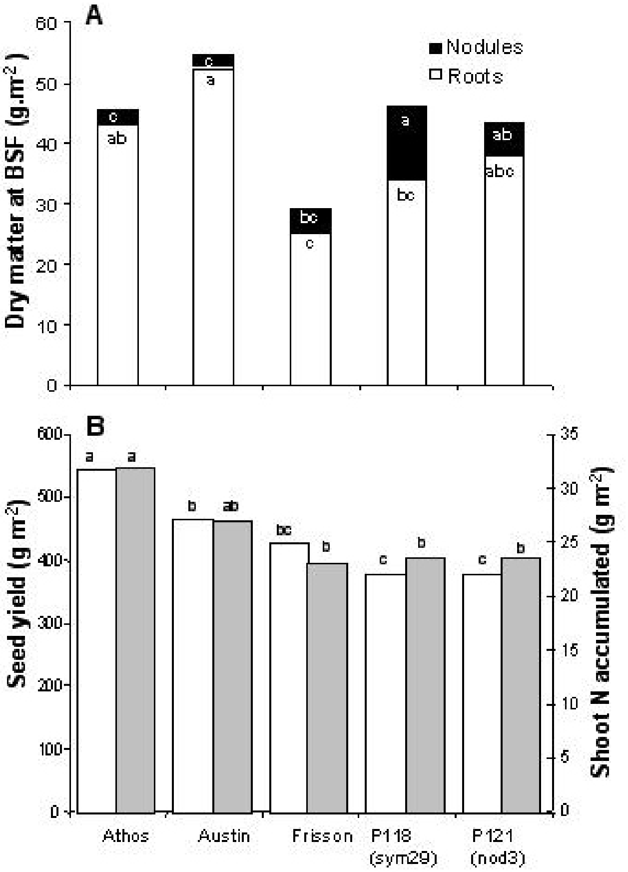

Figure 1. Biomass partitioning between nodules and roots at beginning of seed filling (A) and seed yield and shoot N accumulated at maturity (B), for five pea genotypes experimented in field. Means followed by different letters are significantly different at the 5% probability level.

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required for shoot-controlled of AON (8), whereas *NOD3* acts in the roots, probably before *SYM29* (9). Interestingly, the differences among genotypes in root and nodule development were associated to differences in seed yield and N uptake. In particular, the genotypes with higher nodule biomass produced lower seed yield and acquired less N than the genotypes with higher root biomass (Figure 1).

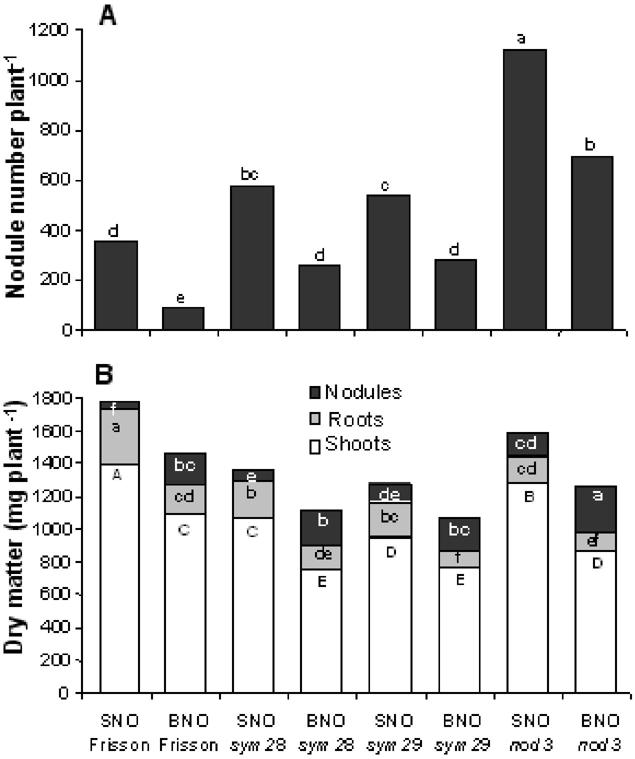

Figure 2. Effect of the rhizobial genotype on nodule development (A) and biomass partitioning between nodules, roots and shoots (B) at beginning of flowering of pea plants of cv. Frisson and its three hypernodulating mutants. SNO, small nodules; BNO, big nodules. Means followed by different letters are significantly different at the 5% probability level.

Genetic variability of the bacterial partner and host preference for particular rhizobial genotypes contributes to the variability in N uptake

The genetic background of the host plant was found to influence the genetic composition of the symbiotic rhizobial populations in nodules (3, 4). The cvs Athos and Austin producing both high root biomass were associated with quite similar *Rlv* populations, which differed from those interacting with cv. Frisson (Figure 2). Such differences of preference for particular rhizobial genotypes were also found between cv. Frisson and its two hypernodulating mutants, with notably lower occurrence of particular rhizobial genotypes in cv. Frisson. These results suggest that the effective AON in wild types could result in a stronger reduction of nodule formation by some rhizobial genotypes compared to others. Furthermore, significant effects of the rhizobial genotype, especially the *nod* haplotype, were found to influence the development of different plant organs (3). Notably, several strains harbouring a particular *nod* genotype induced few but very big nodules (BNO), yielding high nodule biomass, but reduced root and aerial development (Figure 2), as well as reduced N uptake and seed yield. No interaction between rhizobial and plant genotypes was observed: the BNO phenotype was conserved in *sym28*, *sym29* and *nod3* mutants. Moreover, whatever the rhizobial genotype, the *nod3* mutation induced significant higher number of nodules, in agreement with Sagan and Duc (7).

Using an ecophysiological analysis demonstrates that the regulation of N uptake is closely linked to the C metabolism

Our study in field showed interestingly that the amount of N taken up by the plant did not directly depend on the size of the belowground organs responsible for the underlying function; hypernodulating mutants did not fix more N than other genotypes, and genotypes with the highest root biomass did not systematically retrieve the highest amounts of nitrate (Figure 1). Moreover, genotypes with the highest nodule biomass accumulated the lowest biomass in the shoot. A conceptual framework was therefore used to analyse the different abilities of the pea genotypes for N uptake, taking into account its interaction with C metabolism at the whole plant level (5). The variability among genotypes in shoot biomass and N uptake was analysed, considering not only the structures involved in N acquisition in terms of nodule and root biomass, but also their efficiency in terms of N accumulated through symbiotic fixation or mineral absorption per amount of nodule or root biomass, respectively. Especially, nodule efficiency of the genotypes experimented in the field was negatively correlated to nodule biomass. Thus, the high C costs induced by excessive nodule formation/growth in hypernodulating mutants (10) or in all pea genotypes inoculated by BNO strains, presumably limited their activity, and it also limited shoot growth (Figure 2).

The interest of combining plant genetic, ecophysiological and microbiological approaches to enhance N uptake

The ecophysiological analysis of genetic variability for root and nodule development demonstrated that N uptake only partly depended on potential N supply, but was under the control of plant growth. C uptake may especially be a limiting factor of N uptake in the early period of growth, when high competition for C with shoot may limit nodulated roots synthesis and/or nodule efficiency. Moreover, the rhizobial genotypes were shown to influence strongly both nodulated root and shoot development. Due to the high C costs induced by nodule formation and its detrimental effect on shoot and root growth, selecting traits for enhancing N uptake by legumes must be engineered i) considering inter-relationships between C and N metabolisms, ii) considering the diversity of root architectures, iii) in terms of temporal complementarities between symbiotic N_2 fixation and NO_3^- root absorption rather than through direct increase of nodule and/or root biomass and iv) considering the effects of the rhizobial partner.■

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RESEARCH

Manipulating seed quality traits in pea (*Pisum sativum* L.) for improved feed and food

by C. DOMONEY [1]*, A. CHARLTON[2], C. CHINOY[1], H. VIGEOLAS[3], P. GEIGENBERGER[4] and A. CLEMENTE[5]

The promotion of legume crops as significant contributors to sustainable agriculture is linked to crop value and seed quality. Any definition of seed end-use quality traits in pea relates primarily to the various requirements of feed and food industries. Higher seed protein content is desired for animal feed use in some, but not all, EU countries, while the relevance of various anti-nutrients also differs among countries. For food use, there are important traits that may be either specific or common to the use of seeds as immature fresh, canned or mature dried products. It is clear that some so-called anti-nutrients, defined in the context of optimising animal feed, may act as health-promoting agents for humans. The definition of trait genetics, ideally coupled with an understanding of the underlying biochemistry, will provide tools and resources that may be optimised for a broad range of end-uses by the feed and food industries. Marker development facilitates breeding programmes for seed traits in particular, where early screening offers savings on space and costly seed assays.

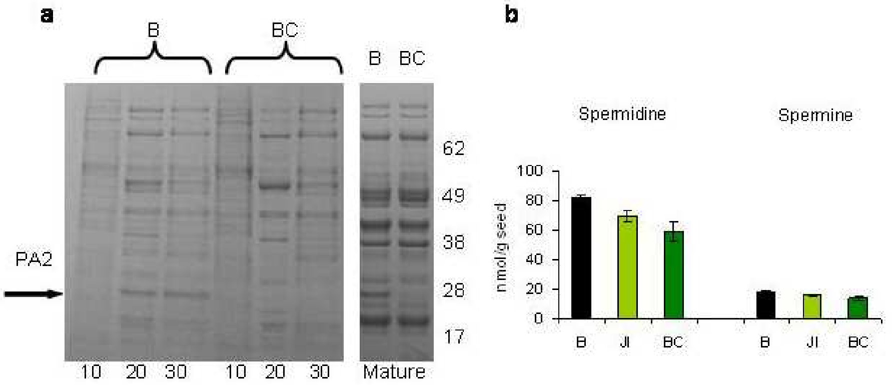

Figure 1: Protein profiles of seeds of *cv.* Birte (B) and BC lines lacking PA2 (arrowed) at 10, 20, 30 days after flowering and maturity (a) and amounts of spermidine and spermine determined for mature seeds of *cv.* Birte (B), JI 1345 (JI) and BC lines (b).

Optimising seed composition for feed use

Several seed proteins have been shown to be poorly digested, or to interfere directly with digestive processes. The pea seed albumin 2 (PA2) and the trypsin/chymotrypsin inhibitor (TI) proteins fall into these two respective classes. PA2 has been shown to resist digestion in piglets and chickens and to persist through the digestive tract (9, 10, 12). A variant line, JI 1345, that lacks PA2 was identified in the John Innes *Pisum* germplasm collection and this null mutation was introgressed into a cultivar (*cv.* Birte) through development of recombinant inbred (RI) and associated backcross (BC) lines (Figure 1a). Studies of these lines showed that a loss of PA2 did not impact negatively on seed protein content, but rather the PA2-deficient lines showed higher seed protein (14). Expression analyses using the Ps6kO-LI1 microarray have identified genes that are more highly expressed in PA2 mutant lines. These genes may be associated more generally with higher seed protein content, and warrant further investigation.

Earlier work has shown that metabolic changes in seed sugar content are associated with alterations in protein amount, including reduced expression of major legumin genes (13). Here, the higher sugar content is a direct consequence of a lesion in a starch biosynthetic gene (2). The elevated seed protein content in PA2 mutant lines was investigated further by metabolite profiling of seeds of *cv.* Birte and the BC PA2-deficient lines during development. Significant changes were noted in a number of metabolic pathways, most notably that leading to polyamine compounds (14). There were clear differences in several individual amino acids: levels of Ala, Phe, Cys, Arg, and Gln were increased, while others, notably Ile, Trp and Glu, decreased in the mutant. A lack of PA2 led to a decrease in the level of spermidine (Figure 1b), while the level of the precursor of spermidine synthesis, Arg, increased. Further analyses of enzymatic activities revealed a lower arginine decarboxylase activity in mutant lines (14). Changes in relative amounts of sugars, organic and amino acids were indicative of an increase in the organic N content and a decrease in the C to N ratio in mutant seeds. The metabolic changes documented for PA2-deficient lines are compatible with a regulatory role for PA2, consistent with structurally related hemopexin-type mammalian proteins. The introgression of the PA2 mutation into breeding material is underway, and field tests will reveal if there is any phenotype associated with this mutation.

The relevance of TI to digestibility of feed has been shown in studies comparing the effects of near-isogenic lines that differ 5-fold in their content of TI (16, 17). Here the coefficient of apparent ileal digestibility was significantly higher when near-isolines having lower TI activity were included in chick diets. Novel variants for TI have been isolated by TILLING, in collaboration with INRA-Evry (http://urgv.evry.inra.fr/UTILLdb). Besides their potential for improving digestibility in animal feed, lines with reduced activity provide tools for examining the physiological role of seed TI and for assessing the contribution of such proteins to human health (see below).

Optimising seed composition for food use

PA2 and the equivalent protein in chickpea (*Cicer arietinum*) can behave as potential allergens in humans (15) and so a reduced content of this protein may provide benefit in diets for certain groups of people, in addition to benefits for improved feed as outlined above.

The positive contribution that TI may make

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to human health as anti-carcinogenic proteins is being investigated. A clear reduction in the growth of HT29 colon cancer cells has been shown when grown *in vitro* in the presence of pea TI (6). These observations gain significance from the demonstration that soybean TI can retain activity following exposure to the digestive tract (7, 11). Although earlier work suggested that the chymotrypsin inhibitory domain may be more effective at inhibiting the growth of cancer cells, recent data show that both chymotrypsin and trypsin inhibitory domains are significant in terms of their effects on cell growth (8). Furthermore, normal fibroblast cells were unaffected by exposure to TI, while inactivated TI proteins showed little or no effect on HT29 cells (8). Serine proteases that are associated with cell membranes have been identified as candidates involved in processes associated with cell migration and metastasis. These include the type II transmembrane serine protease, matriptase (1), where interaction with legume TI warrants further investigation. If validated by *in vivo* studies, legumes genotypes with high TI activity may be selected positively for the development of health-promoting foodstuffs. It is already the case that many of the lines used for marrowfat pea products have a high TI activity but the relationship between this and the marrowfat trait is as yet unknown.

Within the current climate of concerns over food security, health issues may recede in relative importance. However, the definition of compounds involved in the generation of food that is nutritious, desirable and beneficial to health is already benefiting from metabolomic studies that permit simultaneous detection and quantification of many small compounds. Linking these studies to genetic analyses will unravel biochemical and genetic determinants of metabolite concentration. Metabolite analyses have identified downstream changes to pathways and compounds as a consequence of genetic background in pea (14; see above) as well as changes that can be attributed to the environment (4). In the latter study, the environment was shown to have a much greater effect on the leaf metabolome, when compared with the effects of transgenes. The further identification of seed metabolites linked to food quality will provide a guide to pathways and markers for improved selection processes. An example of a metabolite that differs in relative concentration among 22 pea lines is shown in Figure 2.

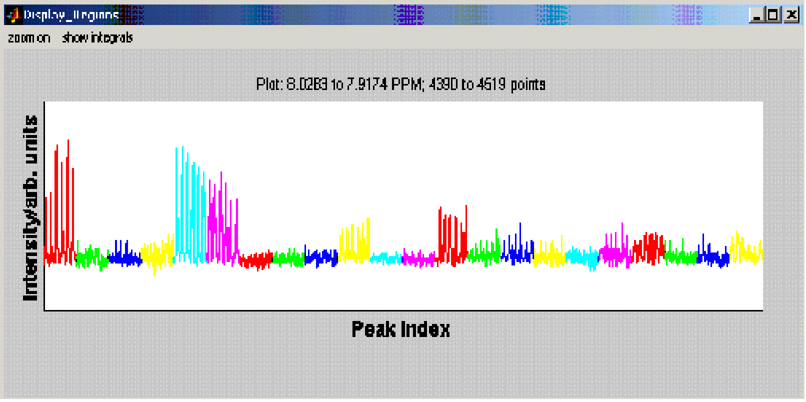

Figure 2: Variation in NMR peak intensity associated with metabolite content in 22 lines of pea, represented by different colours. Six replicate analyses are shown for every line.

The shape, colour and general appearance of vegetables are all economically significant quality parameters, that influence consumer choice, price, return to the farmer, and ultimately crop choices for rotations. These are all particularly relevant to the adoption and extended use of sustainable crops, and efficient nitrogen use. Some seed attributes relate to pest control, for example damage delivered to pea and bean seeds by bruchid beetle larvae, where progress is being made with the genetic basis of resistance (3, 5). Aspects of seed colour are being investigated and variants used to develop RI lines with associated maps to define genetic loci involved in seed colour determination, its stability and degradation (Chinoy, Domoney et al., in preparation). Alongside the selection of novel germplasm, the TILLING platform (see above) is being exploited to identify mutants in candidate genes. The activities of the Pulse Crop Genetic Improvement Network (http://www.pcgin.org) funded by Defra, UK, together with the EU-funded Grain Legumes Integrated Project : (http://www.eugrainlegumes.org) and its associated technology transfer platform (http://www.gl-ttp.com/), are providing a mechanism to foster direct links between this research and the relevant industry.■

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Studies on ascochyta blight on pea in France: Epidemiology and impact of the disease on yield and yield components

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Ascochyta blight is a serious disease affecting field peas, with significant effects on yield and yield components. A field experiment involving "healthy" plots, protected by fungicide applications and with plots artificially inoculated with the fungus, investigated the effect of the disease on yield and yield components (Tivoli et al., 1996). Stem number and height, the number of fruiting nodes and pods were not affected by ascochyta blight. By contrast, the number of seeds per stem, mean seed weight and harvest index were all significantly decreased. The final yield losses observed in diseased plots could be as high as 30 to 40% of the yield obtained from plots with total fungicide protection.

Thus, since the early '90s, we have conducted in France studies on the three elements of the pea/*Mycosphaerella pinodes* pathosystem: the plant, the pathogen and the environment. These elements were considered as essential to understand the epidemic development of the disease and the impact of the disease on plant functioning and yield.

Epidemiology

Onset of ascochyta blight. Schoeny et al (2007) developed a model to predict ascochyta blight onset in field peas based on the calculation of cumulative temperature and moisture indices which were used to define a disease risk forecast window and which relate to airborne inoculum availability. Moreover, Moussart et al. (1998) had shown that when pea seeds were highly infected by *M. pinodes*, the pathogen was detected in the embryo and pycnidia were observed in internal cotyledons. All seedlings developing from seeds infected with *M. pinodes* showed symptoms at or below soil level, and low temperatures were found to increase the frequency of transmission.

Disease development. Under controlled conditions, the onset and development of the disease are tightly linked to temperature: at 20°C, infection is rapid as the fungal spores germinate within six hours and the first symptoms appear within 24 to 48 hours (Roger *et al.*, 1999a). The effects of interrupted wet periods and different temperatures on the development of ascochyta blight were assessed (Roger et al., 1999b). The effects of climatic variables linked to disease development, the formation of fruiting bodies and parasite dissemination have been extensively discussed by Roger and Tivoli (1996). The epidemiological cycle of ascochyta blight on pea showing the role of teleomorph and anamorph forms was described by Tivoli and Banniza (2007). The pathogen spreads by producing pycniospores that serve to establish the fungus on the plant, and ascospores that are responsible for the rapid increase in disease levels after flowering in pea (Roger and Tivoli, 1996). Disease scores measured on different levels of leaves, internodes and pods showed that disease severity increases from the base to the top of plants.

Effect of cultivars on disease epidemics. Disease development depends on the characteristics of the cultivars. Onfroy et al (2007) using the method of point inoculation of stipules, showed that partially resistant pea genotypes limited the coalescence of flecks and lesion extension. These observations were highly correlated with disease severity in seedling tests (Onfroy et al., 1999, 2007). Le May et al. (2009a) compared the epidemic development of ascochyta blight under field conditions of a new type of winter pea (*Hr* genotype) with the classical winter cultivars ‘Cheyenne’ and ‘Dove’. The ‘*Hr*’ genotype had the lowest level of disease and disease was less dependent on sowing date for this accession, as compared to Cheyenne and Dove. In spring pea, the effect of pea canopy structure on ascochyta blight epidemics was investigated in four spring pea cultivars (Bridge, Aladin, Solara, Athos) in two field experiments (Le May et al., 2009b). These cultivars had similar levels of susceptibility to ascochyta blight and presented different architectural features (branching, standing ability, stem height). Canopies with different architecture differed in disease progression on stipules and mainly on pods. Three architectural features acted on disease development: cumulative LAI (leaf area index) and large 'Mean Distance between Nodes' which favoured disease development, and large internode length which reduced the disease severity. Schoeny et al. (2008) investigated the effect of canopy architecture on splash dispersal of the asexual spores of the fungus in controlled conditions, using a rainfall simulator. They have shown that the horizontal dispersal gradient depended on the LAI of the different canopies. The barrier rate, calculated as the ratio between the mean number of lesions assessed on isolated plants to the mean number of lesions assessed on plants in canopies, increased with canopy LAI.

Effect of intercropping on disease epidemics. Schoeny et al. (2009) observed that disease severity on pods and stems was substantially reduced in a pea-cereal intercrop compared to the pure pea crop when the epidemic was moderate to severe. Disease reduction was partially explained by a modification of the microclimate within the intercrop canopy, in particular, a reduction in leaf wetness duration during and after flowering. The effect of intercropping on splash dispersal of conidia was investigated under controlled conditions using a rainfall simulator. Total dispersal was reduced by up to 78% in the intercrop compared

Ascochyta blight symptoms on pea stipules and pods, under field conditions.

Symptoms on detached stipules after inoculation by drop deposit, of spore suspension (*Mycosphaerella pinodes*), under controlled conditions.

to the pure pea stand at full density. These results suggested that cereal plants provide a physical barrier to conidia movement within the intercrop canopy early in the cropping season.

This knowledge in epidemiology was used in several programs of genetics for ascochyta blight resistance in pea (Prioul et al., 2003 ; Tivoli et al., 2006a; Onfroy et al., 2007) or in the model plant *Medicago truncatula* (Tivoli et al., 2006b ; Moussart et al., 2007).

Effects of the pathogen on host physiological processes

The development of symptoms is associated with changes in the physiology of the plant, particularly photosynthetic activity and carbon and nitrogen remobilisation activities.

The disease affects the interception of radiation by causing necrosis and inducing the premature senescence of leaves. It also affects the conversion of this radiation into dry matter, by reducing net photosynthesis. Indeed, Garry *et al*. (1998a) showed that during their interaction the necrotrophic fungus *M. pinodes* markedly reduced photosynthesis in the leaves of a pea plant, and that the magnitude of this decrease increased with the level of infestation. They showed that the effect of *M. pinodes* on photosynthetic activity was twelve times greater than the necrotic area (Bastiaans' coefficient b=12.1). Finally, it diverts some of the assimilates produced by the plant towards the pathogen. Experiments involving plants with various levels of ascochyta blight cultivated in greenhouses showed that the disease also affects the remobilisation of nitrogen from the vegetative organs to the seeds (Garry et al., 1996). Indeed, the concentration of nitrogen compounds in diseased stipules and pods was higher than that in healthy organs, and this difference was particularly marked if the disease was severe: nitrogen seems to be "retained" in the diseased organs and its transfer to the seeds is blocked. This disturbance of both photosynthetic activity and nitrogen remobilisation results in decreased number and individual dry weight of seeds (Garry et al., 1998b; Béasse et al., 1999).

Effect of plant growth stage and plant organs infected on yield

The impact of the disease on yield depends on the growth stage at the onset of disease and the location of the disease on the plant.

Plant growth stage. The indeterminate growth habit of pea plants results in the simultaneous presence on these plants of organs of different types and different ages. This property is of particular importance because the plant response to the disease may differ according to the age of the plant organ affected. Two stages seem to be particularly important for yield development, the beginning of flowering (BF) and the final stage in seed abortion (FSSA) (Garry *et al*., 1996): i) if the plant is infected with *M. pinodes* at flowering, the observed decrease in yield results from a decrease in seed number. ii) if the plant is infected after FSSA, the number of seeds is not affected, and the observed yield loss results from a decrease in individual seed weight.

Location of the disease on the plant. The relative impacts of disease on different organs on plant functioning and yield loss remain unclear. However, Béasse et al. (2000) showed that ascochyta blight occurring on leaves at the base of the plants after canopy closure did not cause yield loss. Indeed, these leaves, which are already old and receive little light at that point, are already beginning to senesce and play no further role in plant growth. Béasse *et al*. (1999) showed that disease on the pods may cause yield loss, but that this loss accounts for no more than 10 to 15% of total yield loss. The remaining loss is due to a decrease in growth due to the decrease in photosynthesis and, in some cases, poor nitrogen remobilisation. Using and building upon a disease-coupled crop growth model published by Béasse et al. (2000), Le May et al. (2005) developed an improved model to predict the impact of ascochyta blight in pea on yield components by incorporating a combination of disease progression in the canopy (number of nodes affected by the disease) and the structure of the canopy (leaf area index profile). Using data from four varieties they showed a good fit between estimated and observed values.

Combining the knowledge acquired on the impact of ascochyta blight on yield in the integrated disease management strategies

Plant pathologists generally adopt one of two distinct approaches for studying aerial diseases appearing in the field and developing in plant canopy: epidemiology stricto sensu (the most frequently used approach) and approaches focusing on damage. For ascochyta blight in pea, a combination of these two approaches has been used in order to integrate epidemiological data providing information on the risk factors for the development of an epidemic into a model simulating the effects of the disease on the growth and development of pea plants. By taking into account disease severity, plant growth stage and the risks of epidemic development, this approach should make it possible to improve the efficiency of fungicide treatments by adjusting treatment timing, thereby reducing the total number of applications. Furthermore, these observations should lead to a better characterization of cultivars in terms of the role of their architecture in the development of disease epidemics and possible their tolerance to the disease, particularly with respect to the ability to maintain normal levels of photosynthesis following infection. This approach, which has rarely been used to date, should make it possible to adapt cropping techniques to the farming characteristics of particular regions (selection of suitable varieties, sowing densities etc.).■

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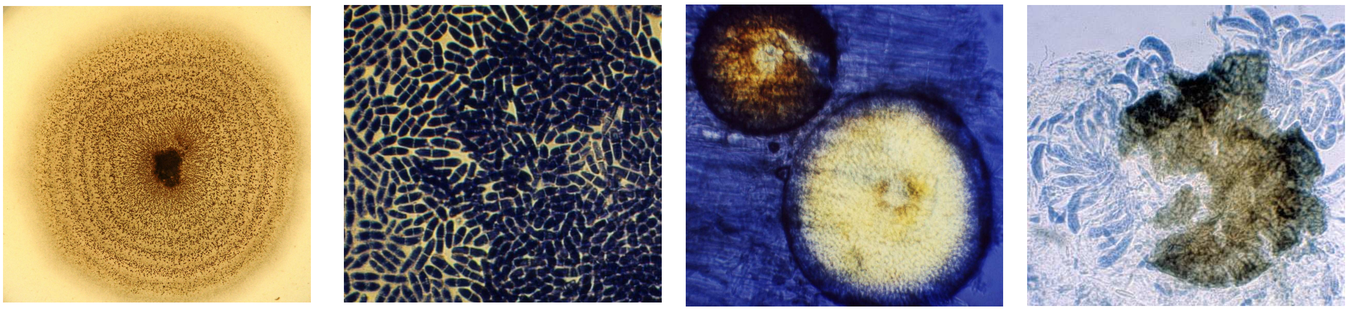

***Mycosphaerella pinodes*: colony in Petri dish (a), pycnidia and pseudothecia (b), pycniospores (c), asci and ascospores (d)**

The pea genetic resources of the Balkans, to represent the first cultivated peas of Europe

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Grain legumes, including pea, had been known to humans before they became cultivated. Together with vetches (*Vicia* spp.) and vetchlings (*Lathyrus* spp.), pea was a part of the everyday diet of the hunter-gatherers at the end of the last Ice Age in Europe, as witnessed by the remains from the site of Santa Maira, Spain, from 12,000–9,000 BC. It seems likely that first domestication of *Pisum sativum* occurred in eastern Mediterranean, Turkey and the Fertile crescent (Fourmont 1956, Smart 1990) before spreading west towards western Europe and east towards Asia. *P. humile* Boiss. et Noë and *P. elatius* Steven ex M. Bieb. are now considered are wild or semi-wild ancestors of *P. sativum* L., these three subspecies constituting a single biological species*Pisum sativum* L. separated from *P. fulvum* Sibthorp et Sm. (Smart 1990).Among the earliest findings of cultivated pea and other grain legumes in the whole world is the site of Tell El-Kerkh, Syria, from 10th millennium BC (Tanno & Willcox, 2006), while in Europe it is worthy to mention the following sites:

- 5790-5630 BC, Kovačevo, southeast Bulgaria;
- 5600-4300 BC, south-western Germany;
- 5470-5260 BC, Aknashen, Armenia;
- 5000-4800 BC until 600 AD, the modern Paris area, France (Bakels, 1999);
- 3900 BC, Lake Constance, southern Germany;
- 3400-3300 BC, Lake Biel, Switzerland.

In all plant species, the process of domestication led to certain morphological changes that, in many aspects, strongly resemble the methods of selection used in contemporary plant breeding programs. In pea, the major criteria to determine the domestication are (1) non-dehiscent pods and absence of seed dormancy, (2) larger seed size and (3) smooth seed testa. The Old World, especially Europe, Near East and Northern Africa were home to various ethnic groups throughout the history. By that reason, common vocabularies of the words related to pea and other grain legumes to both languages within one and the languages of different families, prove that they have been well-known to the ancestors of nearly all modern European nations from time immemorial (Mikić-Vragolić *et al.*, 2007) (see encart).

In the Balkans area, still wild growing *Pisum sativum* subsp *elatius* were reported in botanic books of Serbia and Bulgaria flora of the 20th century and a traditional agriculture has allowed the maintenance of old landraces by local farmers. For these reasons, the French Ministry of Research supported ECONET-EGIDE project 2008-2009 in order to initiate exploration and evaluation of semi-wild and local landraces of *Pisum sativum* from the Balkans and to analyze them in reference to existing national collections of INRA-Dijon-France, VIR- St Petersbourg-Russia, and Sadovo-Bulgaria. In total, the pool of these 3 national collections represents more than 12 000 accessions and each of them is more or less advanced in the definition of a reference- or core-collection.

In botanical books of Serbia, *P. elatius* is stated as native to Serbia and the exploration for new genetic resources has targeted mountain of Homolje, Stara, Planina and Suva Planina. In Bulgaria, according to the "Flora Bulgaricae" v. VI (for the taxons of fam. *Fabaceae*) – *P. elatius* is distributed in dry grassland (and agriculture lands as weed) of all Bulgaria, frequently in the South of Balkan Mountain such as Eastern Rhodopi Mts., Strandza Mts. and South Black Sea (border region with Greece and Turkey). Small groups of 5-10 plants have recently been reported in steppe region of North Bulgaria, in "Kaliakra" reserve and in South Bulgaria near lake "Ivailovgrad" (Eastern Rhodopi). All these explorations are under progress. They already yielded new entries which will be confronted to 80 accessions of *P. sativum subsp. humile* and *P. sativum subsp. elatius* already present in the national collections of the partners of the project.

The development of winter pea varieties which offer the benefit of earlier growth cycle and reduced watering requirements, represent a novelty and a potential in cropping systems for a durable european agriculture. Genetic traits related to winter hardiness (frost and disease resistance) are highly requested and surviving land races are certainly good sources for genes of interest involved in these features. For this reason, the collects of this project mostly targeted winter populations maintained by local farmers. 30 landraces from Bulgaria and 35 from Serbia were already collected. In reference to 60 winter pea accessions from INRA-France and VIR-Russia, they entered in autumn 2009 an evaluation of their frost hardiness in a mountain location in France and in Serbia which will be repeated in 2010.

The next step is now to characterize these new accessions gathered in this project with molecular markers, in order to evaluate their diversity and to establish their historical relationship to wild and cultivated peas. Other traits of interest such as their seed protein composition and disease resistance will also be evaluated. The pea genetic resources of the Balkans, is clearly a valuable source to represent the first cultivated peas of Europe and enlarge the genetic variability available to breeders. ■

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Lexical evidence and traces of *Pisum* species through its domestication

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The majority of the words related to pea in modern European languages belonging to the Indo-European linguistic family originate from several Proto-Indo-European roots, with a subsequent diversification within each of its branches:

- the Latin *Pisum* produced the words denoting pea in numerous modern European languages, such as Albanian, all Celtic languages and nearly all Italic languages, as well as English (Table 1). This word, denoting the same in Latin, has its origin in the Proto-Indo-European **pis-*, meaning *to thresh*;
- the Proto-Indo-European root **erəg*^{w}*(h)-*, denoting a kernel of leguminous plant, brought forth numerous derivations, such the Proto-Germanic **arwait*, denoting pea, from which are derived the words denoting the same in nearly all modern Germanic languages, and the Latin *ervum*, denoting bitter vetch, that gave the words denoting pea in a large majority of Iberian Romance languages, such as Portuguese.
- the words denoting pea in all Slavic languages, including neighbouring languages from another branches of the Indo-European family, have their origin in the Proto-Slavic **gorxŭ*, denoting the same, being itself derived from the Proto-Indo-European **ghArs-*, denoting a leguminous plant in general;
- modern Baltic languages are derived, through the Proto-Baltic **žirn-iã̆*, with the same meaning, from the Proto-Indo-European **g*$^{\prime e}$*r(a)n-*, denoting grain and being the ultimate source of the Latin *granum*, denoting the same.

The most widely form of the words denoting pea in European Uralic languages is, in fact, modified from the words denoting pea in Baltic languages of the Indo-European linguistic family, such as Finnish or Estonian.

The modern Basque word denoting pea, *ilar*, was derived from the Proto-Basque **iłhař*, where it denoted pea, faba bean, vetch and heather.

Both archaeological and linguistic evidence witness that pea has been surely one of the most ancient crops in Europe.

Table 1. Words denoting pea in modern European languages

Family	Branch	Language	Word	Family	Branch	Language	Word
Afro-Asiatic	Semitic	Maltese	*pizella*	Indo-European	Italic	French	*pois*
Altaic	Turkic	Azeri	*noxud*			Galician	*ervelha*
		Turkish	*bezelye*			Italian	*pisello*
Basque			*ilar*			Occitan	*pòis*
Caucasian	East	Ingush	*gerga qeŝ*			Portuguese	*ervilha*
Indo-European	Albanian		*bizele*			Romanian	*mazăre*
	Armenian		*olor*			Sardinian	*pisu*
	Baltic	Latvian	*zirņi*			Spanish	*guisante*
		Lithuanian	*žirnis*		Slavic	Belarusian	*garoh*
	Celtic	Breton	*piz*			Bulgarian	*grah*
		Irish	*pis*			Croatian	*grašak*
		Welsh	*pysen*			Czech	*hrách*
	Germanic	Danish	*ært*			Polish	*groch*
		Dutch	*erwt*			Russian	*gorokh*
		English	*pea*			Serbian	*grašak*
		German	*erbse*			Slovak	*hrach*
		Icelandic	*erta*			Slovenian	*grah*
		Norwegian	*ert*			Sorbian	*hroch*
		Swedish	*ärt*			Ukrainian	*gorokh*
	Greek		*bizéli*	Kartvelian	Georgian	Georgian	*barda*
	Indo-Iranian	Ossetian	*tymbylqædur*	Uralic	Finno-Ugric	Estonian	*hernes*
	Italic	Catalan	*pèsol*			Finnish	*herne*
		Corsican	*pisu*			Hungarian	*borsó*

High throughput identification of *Pisum sativum* mutant lines by TILLING: a tool for crop improvement using either forward or reverse genetics approaches

by C. LE SIGNOR[1], M. DALMAIS[2], V. BRUNAUD[2], R. THOMPSON[1] and A. BENDAHMANE[2]

Several methods are available to researchers or breeders wishing to generate mutations in a plant genome. However, some of these methods are based on plant transformation, and many species, including *Pisum sativum*, remain difficult to transform. Insertional mutagenesis and fast neutron-irradiation techniques have the limitation that they create mainly null mutants of a gene, complicating their use for investigating the roles of essential genes. EMS (Ethyl Methane Sulphate) mutagenesis, which creates single base transitions (G>A) has the double advantage of being suitable for saturating almost all genomes with mutations and for creating allelic series, including weak or potentially advantageous alleles of essential genes. EMS is thus extensively used in forward genetics studies. A recent derivative of this approach, the TILLING technique, has been developed in order to screen a collection of EMS mutants for mutations in a specific gene, i.e. as an EMS-based reverse genetics strategy (Colbert *et al.*, 2001). The completion of the sequencing of several plant genomes has enabled the development of reverse genetics strategies, where one first chooses a target gene based on the functional annotation of its sequence, then proceeds with the identification and phenotypic characterisation of corresponding mutant alleles.

Within the European Grain Legumes Integrated Project, we have created a TILLING collection of 4817 *Pisum sativum* lines mutated with EMS and developed UTILLdb, a database that gathers phenotypic data as well as sequence data of these mutated lines (Dalmais et al., 2008). UTILLdb gives access to phenotypic data based on visual characterization of M2 plants from young seedling to fruit maturation stages. A hierarchical description containing 107 subcategories of mutant phenotypes was used to describe the mutant plants. Currently, out of 4817 M2 families, 1840 were scored with a visible phenotype; among which are the previously described Mendel's phenotypes and previously uncharacterised mutants. UTILLdb is linked with a high-throughput pea TILLING platform which screens the mutant collection for mutations in specific genes. We performed TILLING on 35 genes, identified an average of 10 mutations per kb screened, and estimated the average mutation rate to be one mutation every 200 kb (Table1). The mutation rate found in our population is not significantly different from the rate of one mutation per 300 kb reported for *Arabidopsis* (Greene *et al.*, 2003), rice (Till *et al.*, 2007) and *Caenorhabditis elegans* (1/293 Kb; Gilchrist *et al.*, 2006), and 2,5-fold higher than the rate of two mutations/megabase for Tilling in maize (Till et al, 2004).

Figure 1. Examples of mutant phenotypes representing the major phenotypic groups. (a) Plant 625: stem size : dwarf; leaf shape and arrangements : up-curling and tendrils only; leaf size : narrow; stipule size : narrow; seed size : small, (b) Plant 832: leaf color : glossy; stipule color : glossy; stem size : dwarf, (c) Plant 54: cotyledon colour: yellow bright, (d) Plant 630: flowers: cauliflower type inflorescence; flowers: abnormal all; stem size: dwarf; leaf shape and arrangements: upcurling.

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Genes and mutations were systematically integrated in UTILLdb. UTILLdb may be searched using a sequence, through a BLAST tool, or for a phenotypic feature, by keyword search. The outcome of the search is shown as a table of results that displays the name of each line, with the phenotypes and associated pictures, and mutated sequence if already obtained. UTILLdb is publicly accessible through a web interface (http://urgv.evry.inra.fr/UTILLdb). A link is implemented to facilitate ordering seeds from UMRLEG-Dijon and making TILLING requests to URGV.

To conclude, we have developed a complete tool that can be used for both forward (EMS saturated mutant collection and the associated phenotypic database) and reverse (High throughput Tilling platform) genetics in pea, for either basic science or crop improvement, which is now available to the scientific community.■

Tilled Genes	**Amplicon size (bp)**	**N. Identified mutants**	**N. Screened M2 families**	**Mutation frequency**
Ps CONSTANS-like a (*PsCOLa*)	1012	11	1536	1/141 Kb
Lectin A	971	13	1536	1/115Kb
Sucrose Transporter (*SUT1*)	1014	32	4608	1/146Kb
Cell Wall Invertase (*cwINV*)	1612	35	4608	1/212Kb
Serine-Threonine proteine kinase (*Sym29*)	2457	8	768	1/236Kb
Phosphoenolpyruvate carboxylase (*PepC*)	1009	25	3072	1/124 Kb
Lec1-like (*L1L*)	870	21	4608	1/191Kb
DOF Transcription factor 2 (*PsDOF2*)	1200	9	3072	1/410Kb
Subtilisin	978	31	3840	1/121Kb
Trypsine inhibitor (*TI1*)	712	13	3840	1/210Kb
Pea Albumin (*PA2*)	746	9	3072	1/255Kb
Anther specific protein (*End1*)	851	31	3072	1/84 Kb
MADS box gene (*PM10*)	1302	20	4608	1/300 Kb
MADS box gene (*PM2*)	1390	28	4608	1/229Kb
MADS box gene (PM9)	1072	17	4704	1/297Kb
Transcription factor (*TL*)	1104	28	3072	1/121 Kb
Eukaryotic translation Initiation Factor (*eiF4e*)	1383	36	4608	1/177Kb
Eukaryotic translation Initiation Factor (*eIF (iso)4e*)	772	10	4608	1/356Kb
Methyl transférase 1 (*Met1*)	3842	96	4704	1/188 Kb
Retinoblastoma related (*RBR*)	2959	72	4608	1/112 Kb
Late embryogenesis abundant protein (*PsLEAM*)	952	17	4608	1/258Kb
Heat shock protein 22 (*HSP22*)	622	18	4608	1/159Kb
Mitogen-activated protein kinase 4 (PsMPK4)	1402	37	4704	1/178Kb
Dicer-like 2 (DCL2)	3474	44	4608	1/364Kb
Dicer-like 3 (DCL3)	3171	64	4608	1/228Kb
Cup-Shaped Cotyledon 3 (PsCUC3)	1404	26	4704	1/254Kb
No Apical Meristem3 (PsNAM3)	1216	37	4704	1/155Kb
More Axillary Growth (PsMax1)	1298	18	3072	1/221Kb
Teosinte branched (PsTb1)	933	12	4704	1/366Kb
PsFVE	1227	23	4704	1/251Kb
Afila Zinc Finger 1 (PsAFZF1)	468	21	4704	1/104Kb
Afila Zinc Finger 2 (PsAFZF2)	474	17	4704	1/131Kb
LOB	844	18	4704	1/221Kb
Pheophorbide a Oxygenase (PsPAO)	1330	34	4704	1/184Kb
Rubisco small subunit (RbcS-3A)	1288	12	4704	1/505Kb
TOTAL/MEAN	47359	467	-	1/217Kb

Table 1 : Tilled genes and mutation density in the Cameor mutant population.
The entire Cameor mutant population, or part of it, was screened for mutations in the genes indicated on the left of the table. The size of the screened amplicon, the number of mutants identified and the mutation frequency for each amplicon are indicated. The average mutation frequency was estimated to one mutation each 200 kb.

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Challenges for forage pea breeders

by V. MIHAILOVIC[1], T. WARKENTIN[2], A. MIKIC[1] and B. ĆUPINA[3]

Pea (*Pisum sativum* L.) is one of the most important legume crops in the world. It is characterized by a wide variability of morphological traits and thus is able to be used in many diverse ways. Although this characteristic is present in many other annual legume crops such as grass pea (*Lathyrus sativus* L.) and other vetchlings (*Lathyrus* spp.), vetches (*Vicia* spp.), faba bean (*Vicia faba* L.) or lentil (*Lens culinaris* Medik.), it could be said that it is most prominent in pea, greatly progressed and accelerated by various breeding programmes.

The forms forage pea is used

Among the many ways of utilization of the pea crop are those related to forage production. Green forage represents the above ground parts of a plant cut at full flowering or at the formation of the first pods, which represents a balance between yield and quality. Forage dry matter is what remains from green forage after a proper drying until more or less constant mass in field conditions, with most part of leaves preserved. Forage pea may be used in the form of meal, similar to lucerne or clover forage, as well as silage, together with acidifiers, and haylage, as a special way of forage self-conservation. Regardless of the specific way of use, forage pea may be cultivated alone, as a pure crop, or in mixtures with cereals, such as barley, oats, triticale, rye or wheat, which represents one of the most traditional ways of production in many regions of Europe. In the case of the latter, in Serbia it is always necessary to cut a mixture before the cereal component shoots out its spike, while in western Canada, pea-cereal mixtures are typically cut when the cereal crop reaches the soft dough stage. In more recent times, several attempts have been made in Novi Sad to cultivate forage pea in mixtures with other annual legumes such as faba bean or white lupin, where pea would play the role of a supported crop, with a main goal of increasing the total forage protein yield, with the preliminary results available soon.

The potential of pea for forage production

The results of a long-term trial in Novi Sad, including tens of cultivars of several most important annual forage legumes, shows that, on average, forage pea may be considered with a great potential for both forage dry matter and forage crude protein production (Table 1). Similar forage dry matter yields and crude protein contents have been obtained in spring forage pea cultivar trials in western Canada over the past five years.

In temperate regions, there is a clear difference between winter and spring cultivars. On the other hand, only spring sown cultivars are produced in northern regions with extremely harsh winters, while winter-sown cultivars are preferred in the Mediterranean climates. A long-term trial in Novi Sad showed that, on average, winter cultivars of forage pea has higher yields and better quality, that is, higher forage protein yields, than spring cultivars. However, the latter have a much more prominent ability to form a considerable above-ground biomass for a brief period of time in comparison to the former.

What a forage pea cultivar should look like?

The main goal of forage pea breeding programs is high quality and stable forage yields. In most cases, this means green forage yield of more than 50 t ha^{-1} in winter and more than 45 t ha^{-1} in spring cultivars, with an average forage dry matter proportion of more than 22%, leading to the forage dry matter yields of about 10 t ha^{-1}. An average variation of forage yields between years is aimed to be less than 20%. At the same time, a modern forage pea cultivar must have reliable seed yields in order to survive in the market.

Regarding the fact that total yield represents the contribution of all yield components and although increased plant height is often associated with high forage yield, it is the number of internodes that has a more positive impact on it, since it has a high positive correlation with number of leaves. This trait is regulated by a number of dominant genes and is easy to introgress. At the same time, forage yields can be improved by a moder-

Species and type	Green forage yield (t ha^{-1})	Forage dry matter yield (t ha^{-1})	Forage crude protein yield (kg ha^{-1})
Winter pea	46	9.1	1775
Spring pea	40	8.2	1542
Spring grass pea	43	8.5	1827
Winter common vetch	39	7.9	1651
Spring common vetch	34	7.5	1567
Winter Hungarian vetch	32	7.7	1509
Winter large-flowered vetch	29	6.4	1350
Winter hairy vetch	41	8.4	1764
Winter bitter vetch	33	7.9	1659
Spring Narbonne bean	27	5.7	1362
Spring faba bean	48	10.2	2071
Spring lentil	21	4.3	860
Spring white lupin	44	8.3	2241
Cowpea	36	7.1	1491
Soybean	42	8.3	1909

Table 1. Potential of forage pea and some other annual legumes for forage production in a long-term trial in Novi Sad

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ately prominent basal branching. The most common ideotype of a forage pea cultivar is unlike the one of a grain pea cultivar (Figure 1a).

Forage pea quality is in direct positive correlation with the proportion of leaves in the total forage yield, since it is leaves that contribute most to forage protein yield. A classical forage pea cultivar has large stipules and two or, if possible, three pairs of large leaflets. An alternative solution may be the introduction of forage pea cultivars with acacia leaf type (Figure 1b), with a large number of leaflets and without tendrils, with an expected increase in leaf proportion and better quality (1). However, these cultivars are extremely prone to lodging, and thus may severely suffer either from low seed yields or large seed losses during harvest, making their commercialization questionable and leaving them suitable only for mixtures with small grains.

Reliable seed yields are requested in all modern annual forage legume cultivars, thus a cultivar, apart from high quality and stable forage yields, is able to produce medium or high seed yields in order to make its commercialization successful. In our teams, a strong emphasis is put upon smaller seed size, preferably less than 200 g per 1000 seeds, with double benefit: an increased number of seeds per plant, and thus higher coefficient of multiplication, and a less expensive sowing for the farmers. A novel approach to this issue brought forth semi-leafless forage pea cultivars (Figure 1c), where a plant height up to 100 cm and afila leaf improve standing ability, a greater number of internodes and large stipules provide high and quality forage yields, thicker and more juicy stems make forage more palatable for ruminants, and pods grouped in the upper half of a plant increase seed yields (3).■

Figure 1. Various ideotypes of a forage pea cultivar: (a) classical (left), in comparison to a typical grain pea cultivar; (b) acacia-leaved; c) semi-leafless.

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Morphological and molecular relationships in *Pisum* L.

by P. SMYKAL[1], G. KENICER[2], A.J. FLAVELL[3], O. KOSTERIN[4], N.T.H. ELLIS[5] and M.J. AMBROSE[5]

Classification of *Pisum* is wonderfully complex and has produced a great many names reflecting its importance since Neolithic times. Early morphological classifications of *Pisum* included species such as *P. maritimum* (now *Lathyrus japonicus*), and *P. formosum* (now *Vavilovia formosa*). Improvements in karyology and microscopy refined the view of the genus over the past century to give an apparently natural monophyletic group (i.e. one with an exclusive common ancestor). Recent leaps forward in molecular DNA-based data have helped clarify our understanding of *Pisum* phylogeny (the study of relationships), but not necessarily made it any simpler.

Morphology-based classifications have suggested a genus with five species (Govorov 1937), a monotypic genus (Lamprecht 1966, Marx 1977), and a genus with two species (Davis 1970), as currently most commonly accepted. The two species are *P. fulvum* Sibth. & Sm. and *P. sativum* L. The more recent classification of Maxted and Ambrose (2000) adopted three species:

- *Pisum sativum* L.
    - subsp. *sativum* (includes var. *sativum* and var. *arvense*)
    - subsp. *elatius* (Bieb.) Aschers. & Graebn (includes var. *elatius*, var. *brevipedunculatum* and var. *pumilio*)
- *Pisum fulvum* Sibth. & Sm.
- *Pisum. abyssinicum* A.Br.

Nomenclature in the group is complex, with many additional names having been used at species and infraspecific levels. *Pisum humile* was described by Boissier and Noe (1856), a name used earlier by Miller (1768) for a form of cultivated pea. Berger (1928) downgraded the rank to subspecies and gave it new name: *P. sativum* subsp. *syriacum* (Boissier and Noe) Berger. Makasheva's (1979) classification recognised *P. fulvum* and a *P. sativum* complex (with subspp. *sativum, elatius, syriacum, asiaticum, abyssinicum, transcaucasicum*) (Figure 1). Ben-Ze´ev and Zohary's (1973) work on crosses suggested '*elatius*' and '*humile*'='*syriacum*' are ecological morphotypes (tall forms of woods and maquis versus low forms of grasslands and field weeds, respectively). The domestication of cultivated pea from northern populations of *P. humile* was proposed by Ben-Ze´ev and Zohary (1973).The clear-cut phenotype and karylogical barrier to crossing, support the view of *P. abyssinicum* as a distinct species. Lehman (1954), and later Green (2008), proposed an artificial but functional classification based on combinations of four simply inherited characters to give six functional types.

Pisum phylogeny based on molecular data

With advent of molecular methods, analysis of seed protein (Waines 1975), chloroplast DNA polymorphism (Palmer et al. 1985) and nuclear ITS sequence variation (Polans and Saar 2002) led taxonomists to consider *P. fulvum* as a distinct species and *P. sativum* to be an aggregate of *P. humile, P. elatius* and *P. sativum*. Within this aggregate, *P. humile* is considered to be the closest wild relative and the direct progenitor of cultivated pea. Phylogenetic studies based on retrotransposon markers support the model of *P. elatius* as an paraphyletic group, within which all *P. sativum* is nested (Ellis et al. 1998, Pearce et al. 2000). *P. fulvum* and *P. abyssinicum* formed neighbouring but separate branches, while *P. elatius* was positioned between *P. fulvum-P. abyssinicum* and cultivated *P. sativum* (Vershinin et al. 2003, Jing et al. 2005). The extremely low diversity of *P. abyssinicum*, could be explained by passage through a bottleneck, associated with a putative hybridization event between *P. fulvum* and *P. sativum*. All these findings indicate frequent recombination events between multiple ancestral lineages and the impact of introgression on pea diversity and evolution, as further supported by analysis of 39 gene-derived intron fragments (Jing *et al*. 2007). A combination of mitochondrial, chloroplast and nuclear genome markers was used by Kosterin and Bogdanova (2008) resulting in separation of *P. fulvum* and *P. abyssinicum* accessions and about half of those of wild *P. sativum* from the rest of the wild and all

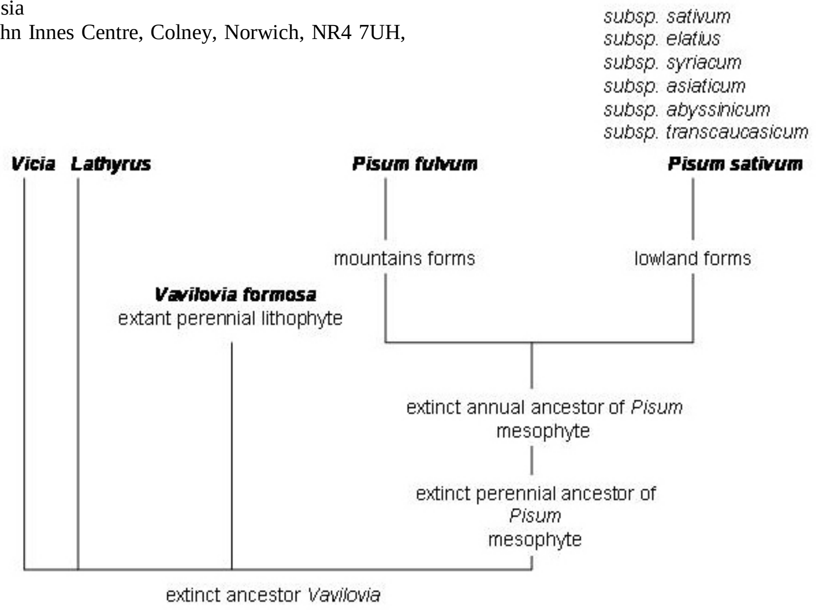
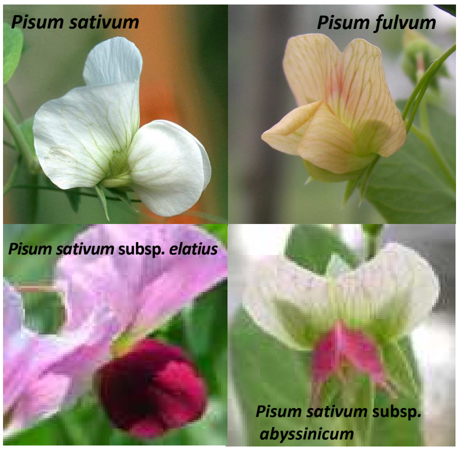


Figure 1: Hypothetical origin of *Pisum* according to Makasheva (1979).



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cultivated *P. sativum* (Figure 2). The capture of mutation transitions in three unrelated markers from different genomes highlights the divergence within *P. sativum* ssp. *elatius* that gave rise to part of wild *P. sativum* and consequently the cultivated ones. In fact, comparison of results between different phylogenetic analyses is limited and difficult due to differences in studied accessions as well as markers. Moreover, incomplete information on taxonomic attribution and origin of wild accessions hinder such studies.

Pisum within tribe Fabeae

Studies attempting to position *Pisum* relative to other genera in tribe *Fabeae* are limited, as most phylogenies in the tribe restrict their sample sets to within genera, making assumptions of monophyly, and use only a few species from outside. Based on floral (stylar) characters and molecular data (ITS and chloroplast genes) *Pisum* has been shown to sit between *Vicia* and *Lathyrus* (Kenicer et. al 2005, Endo et. al 2008). It certainly appears that *Pisum* should not be thought of in isolation. It is undoubtedly nested in *Vicia*, perhaps in *Lathyrus* as well, and is very closely allied to *Vavilovia* (Smýkal et al. 2009). Much more comprehensive sampling of species throughout tribe Fabeae should provide a far better understanding of this taxonomically complex grouping. ■

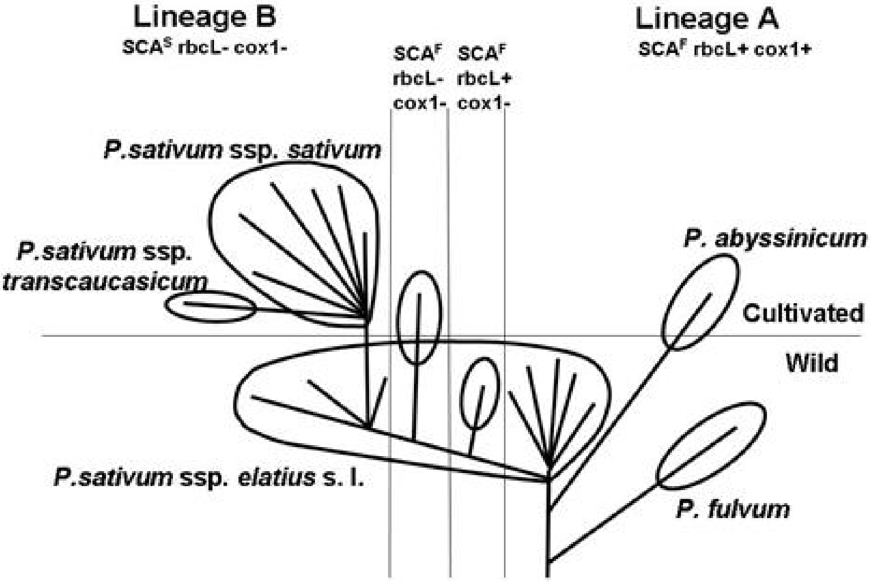

Figure 2: Hypothetical scheme of phylogeny of the genus *Pisum* inferred from plastid, mitochondrial and nuclear genome markers, according to Kosterin and Bogdanova (2008)

P.S. acknowledges financial support from the Ministry of Education of Czech Republic LA08011 project.

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Disease resistance in pea

by D. RUBIALES[1] D, E. BARILLI[1] and S. FONDEVILLA[2]

The adaptability and productivity of pea (*Pisum sativum* L.) are limited by major diseases. Breeding for disease resistance involves diagnosis, development of efficient screening methods, availability of resistance sources, and the development of effective breeding methodology. Major genes conferring resistance to several pea diseases have been identified but unfortunately no efficient sources of resistance have been described to date to the most important pea diseases (broomrape, ascochyta blight, Aphanomyces…) or it is scarce and of complex nature, making necessary the implementation of other control measures. Still, significant genetic variation for these traits exists for pea.

The most important root diseases are fusarium root rot (*Fusarium solani* f.sp. *pisi*), fusarium wilt (*F. oxysporum* f.sp. *pisi*), aphanomyces root (Aphanomyces *euteiches*) and the parasitic weed broomrape (*Orobanche crenata*). Resistance to *F. solani* is quantitatively inherited. Three QTL associated with resistance and STMS markers for use in marker assisted breeding have been reported (2). Single dominant genes have been reported for the various known races of *F. oxysporum*, gene *Fw* to race 1, *Fwn* to race 2 and *Fwf* to race 5. Gene *Fw* is bred into most cultivars grown currently (7). Genetic resistance to *A. euteiches* in pea is known to be quantitative and largely influenced by interactions with environmental conditions. QTLs have been identified associated with partial field resistance (9). Only incomplete resistance to *O. crenata* is available in pea germplams (13). Four genomic regions associated with field resistance and several QTL governing specific mechanisms of resistance “in vitro”, such as low induction of *O. crenata* seed germination, lower number of established tubercles per host root length unit, and slower development of tubercles have been identified (6).

Major aerial diseases are ascochyta blight complex (*Ascochyta pisi*, *Mycosphaerella pinodes* and *Phoma medicaginis*), powdery mildew (*Erisyphe pisi*), downy mildew (*Peronospora viciae* f.sp. *pisi*), rusts (*Uromyces pisi* and *U. viciae-fabae*), bacterial blight (*Pseudomonas syringae* pv. *pisi*), Pea Seed-borne Mosaic Virus (PSbMV), and Pea Enation Mosaic Virus (PEMV). Some levels of incomplete resistance against *M. pinodes* and *P. medicaginis* have been reported and QTLs identified (5, 11, 15, 16). Three genes for resistance to powdery mildew, named *er1*, *er2* and *Er3* have been described so far. Only *er1* gene is in wide use in pea breeding programs. Expresssion of *er2* gene is strongly influenced by temperature and leaf age. *Er3* gene was recently identified in *P. fulvum* and has been successfully introduced into adapted pea material by sexual crossing (4). Only incomplete levels of resistance not associated with hypersensitivity have been reported to both rust species and preliminary mapping studies initiated (1, 17). Race-specific resistance is known to downy mildew but there are evidences of breaking down of this resistance. Partial resistance is also known (14).

Single dominant genes are known for resistance to *Pseudomonas syringae*, gene *Ppi1* to race 2, 3, *Ppi3* to race 3 and *Ppi4* to race 4. Resistance to all races including race 6, for which there are no known commercial resistant cultivars is available in *Pisum abyssinicum* (3). Several recessive resistance genes to PSbMV have been identified (*sbm1*, *sbm2*, *sbm3* and *sbm4*) (11). Hence many commercial varieties of pea remain susceptible to the more common strains of PSbMV. Resistance to PEMV is conferred by a single dominant gene (*En*) and has been incorporated into recently released varieties (8).

Resistance conferred by major genes have been incorporated into pea varieties. However, for most important diseases, only quantitative resistance is available. Although QTLs controlling quantitative polygenic resistance have been identified, the distances between the flanking markers and QTLs are still too high to allow and efficient marker assisted selection. Also, accuracy of phenotypic evaluation should be improved as it is of the utmost importance for accuracy of QTL mapping. Dissecting of the resistance into specific mechanisms would improve the accuracy of disease screening and could contribute to refine the position of the QTLs and identify molecular marker more closely linked to the resistance genes. However, available information on responsible mechanisms is still scarce for most pea diseases. In addittion, comprenhensive studies on host status and virulence of the causal agents are often missing. Only after significant input to improve existing knowledge on biology of the causal agents and on plant/pathogen interaction, resistance breeding will be efficiently accelerated.

Broomrape: Resistant accession surrounded by susceptible ones

Effectiveness of MAS might soon increase with the adoption of the new improvements in marker technology together with the integration of comparative mapping and functional genomics. Major progress is likely to be made from the use of *Medicago truncatula* as models for pea disease studies, since the genomics effort is greater in these species compared to pea, mainly due to a much smaller genome. *M. truncatula* is affected by many of the pathogens and pest limiting pea yield. In most cases, screening of germplasm collections of *M. truncatula* allows identification of a wide range of differential responses to the pathogen from highly susceptible to resistant. This serves as base for the characterisation of

Powdery mildew: Resistant *vs* susceptible pea lines

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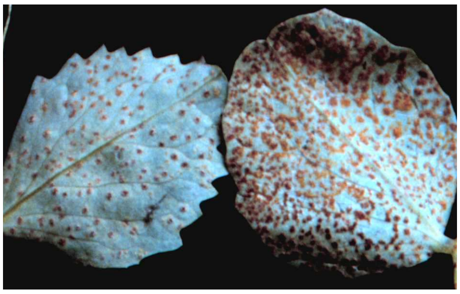
underlying resistance mechanism at cellular and molecular level as well as identifying defence genes and QTLs responsible for resistance. In parallel, the transcriptomic and proteomic approaches developed for this model legume are being used to understand the molecular components and identify candidate genes involved in *M. truncatula* defence against these pathogens (12, 13). ■



Rust: partially resistant *vs* susceptible pea lines

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Environmental benefits of grain legumes in cropping systems

by M.H. JEUFFROY[1], G. CORRE-HELLOU[2], A. AVELINE[2], B. CARROUEE[3] and A. SCHNEIDER[3]

Increasing agricultural production is generally a desirable goal in the context of an increasing World human population. The intensification of agricultural practice during the second half of the XXth century, with more inputs (such as nitrogen, pesticides, irrigation) allowed significant yield improvement in most arable crops. However, at the same time, these types of farming systems, together with other human activities, have contributed to many negative environmental impacts leading to several risks or damages:

- greenhouse gas emissions: arable crops are responsible of half of the world N_2O emissions issued from human activities (Smith 2006); industrial nitrogen fertilisers are usually the major source (70 to 90%) of N_2O emission linked to an agricultural product;
- a consumption of fossil energy, contributing to resource scarcity; this is also mainly linked to the use of more nitrogen fertilizers (fertilisation represents more than 50% of the energy consumption in the farm systems based on arable crops);
- the high use of pesticides, leading to contamination of water, air and soils (Aubertot et al., 2006) with risks of toxicity,
- The use of increasing amounts of water for irrigation contributes to the depletion of underground water.

Since nitrogen use in arable lands is the cause of several major environmental problems, and since nitrogen is needed by all arable crops, except legumes, these plants are of interest because they fix atmospheric in symbiotic association with Rhizobia. A legume crop fixes about 100 to 250 kg N ha^{-1}. The absence of nitrogen fertilizer on the legume crop and the reduction of fertilizer amount required by the following crop, due to highly efficient nitrogen mineralisation, allows a significant reduction of fossil energy consumption and of greenhouse gas emissions. For example, in the Barrois region in France or Saxe-Anhalt in Germany, Nemecek et al. (2008) showed a reduction of (i) 50% for energy consumption of a pea crop compared with oilseed rape, wheat or barley crops, leading to an average reduction of 13% for a rotation which includes 20% of legumes; (ii) 50% for the greenhouse gas emissions for the pea crop compared with the other crops, and of 14% for a rotation including pea, compared with the rotation without pea; (iii) up to 18% for the acidification per hectare of the rotation including pea compared to the reference one without pea.

Another benefit of the legumes is linked to the diversification of the crop successions and the consequent effect on weeds, pests and diseases. In Europe, successions involve a high proportion of cereals (Mignolet et al., 2004), this proportion has been increasing for 30 years and it has been shown that the frequency of occurrence of soil-borne diseases, such as eyespot (Colbach et al., 1996), is decreased in rotations including pea or oilseed rape as compared to the rotations based on wheat and maize: in comparison with a maize-wheat rotation ploughed each year, the occurrence of the disease is decreased by 54% on a rotation oilseed rape – pea – wheat. The same effect has been observed on weeds: when the rotation involves a spring pea, the weed density is decreased from 11 to 0.01 pl.m^{-2} for ploughed conditions, and from 706 to 3 pl.m^{-2} for unploughed fields (Chauvel et al., 2001).

Furthermore, soil structure under a crop varies depending on which was the previous crop. When the preceding crop is harvested in wet conditions in the autumn (maize and sugar beet), the compacted zones can represent more than 20% and up to 50% in average, compared to only 15% in the case of pea which is harvested in early Summer (data from a 8-years experiment in Mons, France, Vocanson et al., 2004).

Finally, water requirements for a pea crop are lower than for soyabean (50% lower) or maize (66% lower) (Munier-Jolain & Carrouée, 2003), resulting in a lower irrigation requirement (from 50 mm on pea in average, compared to 200 and 250 mm in soybean and maize respectively, average data from South-West France).

Nevertheless, the risk of nitrate leaching during the winter is often higher after a pea crop than after other crops such as barley or wheat (Jensen *et al.* 2004, Carrouée *et al.*, 2006). This is mainly due to a higher amount of mineral nitrogen available in the soil (average +30 kg N.ha^{-1}) at the beginning of winter, the start of the drainage period. This higher amount of N in the soil during autumn is linked to a shallower root system and to the dates of sowing/harvest (the short plant cycle with an earlier stop of mineral nitrogen absorption), leading to higher mineral N at harvest. This negative environmental impact of legumes can be reduced by sowing a catch crop just after pea harvest: this allows a large reduction of nitrate leaching risks. Moreover, on-going research is targeted to breed cultivars with a deeper and more efficient root system. Interestingly, there are some results which show that, the second autumn after cropping, the amount of mineral N in the soil is lower after a legume than other crops (Hauggaard-Nielsen 2003; Thomsen 2001).

The high mineral N availability offered by a grain legume crop allows a reduction of the amount of N-fertilizer to be applied on the following crop (average of 20-40kg.ha^{-1}, on-going research). On this topic, some progress can be achieved on the farms: farmers are often not taking into consideration all the benefits of pea as a preceding crop. For example, N-fertilizer application on wheat varies very few according to the preceding crop (CERFRANCE data on farmers' practices).

Finally, the environmental benefits of legumes could be enhanced by introducing several innovations in the cropping systems. First, the pea is most often grown before a wheat crop, as there are many advantages for this succession. Yet, it seems to be possible to reinforce the preceding crop effect by growing an oilseed rape after pea. In fact, this crop is able to uptake large amounts of nitrogen during autumn, reducing the risk of nitrate leaching after pea, and the saving in N fertilisers is even more beneficial for these high N consuming rotations. Another interesting innovation is intercropping, i.e.sowing and harvesting together a cereal and a legume in the same field. Numerous studies have shown the advantage of these intercrops: higher yields and grain protein content, production stability, better nitrogen use efficiency, reduction of nitrate leaching risk, lower use of pesticides and better energy balance (Yvergniaux et al., 2007).

In conclusion, it is urgent to change cropping systems in order to achieve the environmental requirements of a more durable development. However, the evolution of cropping systems requires the involvement of all the stakeholders and actors influencing agricultural practices. Thus the design new cropping systems requires the involvement of a diversity of actors and the implementation of participatory methods.■

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Field Pea: a cover crop in establishing perennial legumes

by B. CUPINA[1], A. MIKIC[2] and D. KRSTIC[1]

Perennial legumes can be sown either in the summer, autumn or spring. As small seeded legumes are sown shallowly and are particularly vulnerable to drought stress, lucerne and red clover are generally sown in spring in Serbia when conditions are more favorable for establishment; however, a spring-sown crop has a significantly lower yield in the year of establishment than a crop sown in the previous autumn. Furthermore, weeds are a much greater problem in a spring-sown crop (Cupina *et al*., 2000). An annual legume forage or grain crop when sown as the companion can provide an economic yield during establishment of the perennial forage crop and produce a return in the seeding year (Tan *et al*., 2004). Zollinger and Meyer (1996) reported that pure crops of perennial legumes produced significantly lower forage yields than intercrops and had slower growth during establishment that increased their vulnerability to weed invasion. Intercropping with short-season crops, such as field pea, significantly reduced incursion of weeds. The proportion of the annual yield derived from the first cut in intercropping with field pea reaching up to nearly 80% and the annual trends in yield followed closely those at the first cut. Furthermore, undersown crop yields at subsequent cuts during the establishment year were not reduced by the field pea companion crop, since pea has suitable morphological and biological characteristics, which tend to balance the negative effects it may have as a cover crop in both the establishment and first full harvest year. In Serbia and its Vojvodina province small grain, primarily oats and barley are traditionally intercropped with perennial legumes but these tend to be too fast-growing and too competitive for the legume (Cupina *et al*., 2000). Information on alternative companion crops is limited.

Success of companion cropping depends on the capacity of the undersown crop to develop in the shade of the companion crop, because the competition for nutrients, light and water may reduce the yield and resistance of the nurse crop, especially if planted with small grains. Field pea (*Pisum sativum* L.) is suitable for intercropping with perennial legumes because the crop can be harvested quickly and the canopy structure is not dense enough to cause suppressive shading. In addition field pea may act as an additional source of nitrogen for the undersown crop. Because of its short growing season, field pea is suited to be harvested when the first (establishment) cut of perennial legumes is due, increasing protein yield significantly in the harvested crop. Faulkner (1985) found normal-leafed pea cultivars prone to lodging, suppressing growth of the undersown species. Appropriate field pea cultivars should be chosen with care for intercropping since this will affect the risk of lodging, which in turn will affect the likelihood of soil contamination of harvested herbage or degree of suppression of the undersown crop. Cultivars of field pea currently available differ in morphology, primarily in leaf structure and plant height. Afila type peas with short stems and reduced laminae, resulting in more developed, tendrils, are important for intercropping as light penetration is much better, providing better conditions for initial growth to the undersown crop. According to Simmons *et al*. (1995) light intensity at the level of the perennial legume within the semi-dwarf companion crop canopy was consistently higher than with the conventional-stature companion crops.

In addition to selection of a suitable field pea cultivar as a companion crop, appropriate cropping practice also needs to be developed. To mitigate the effect of competition among the intercropped plants, it is recommended that the normal seeding rate of the companion crop be reduced (Vough *et al*. 1995), what requests that the optimum stand density, i.e., the number of plants of the companion crop per unit area, needs to be determined (Tan *et al*. 2004). Dense and lodged companion crops can interfere with the undersown crop, resulting in thin stands. Companion crop competition may be partially reduced by cultural practices, such as reducing the companion crop seeding rate and cutting as early as possible being generally recommended. To avoid too severe competition to the undersown legume, it has been recommended that the sowing rate of the companion crop should be reduced by up to half (Vough et al. 1995). Also, if the cost of pea seed is taken into account, it is reasonable to question the use of the highest seeding rate. Once these criteria are met, then it should be possible to produce a crop that will be high yielding, nutritious and palatable to most livestock. ■

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Wild pea, *Vavilovia formosa* (Stev.) Fed.(*Fabaceae*) in-situ investigation in Armenia

by N.G. SARUKHANYAN[1], J. A. AKOPIAN[2], I. G. GABRIELYAN[2] and A.G. VANYAN[3]

In Armenia, open-environment studies on the wild perennial pea *Vavilovia formosa* (Stev.) Fed. started in late thirties of the past century. On the territory of Armenia, for the first time *V. formosa* was collected on the slopes of Kapujukh Mountain, in the alpine zone of the Zangezur Mountain Range, during the expedition launched by the Nature and History Museum of Armenia in 1929. After that, in 1937, in the same area the plant was found by An. Feodorov (2). Another native habitat of *V. formosa* is located in Central Armenia, in the Geghama Mountains. The species was found in the top area of the volcanic mountain of Sev Sar and in the region of crater Lake of Aknalitch by Y. Kazaryan in 1929 and 1935, followed by D. Sosnovskyi, A. Maghakyan, A. Fedorov, and more recently by A. Akhverdov, N. Mirzoeva, S. Tamamshyan, Y. Mulkijanyan, and E. Gabrielyan. As Y. Kazaryan mentioned (quoted from Fedorov, 1936), at that time, on the slopes of Sev-Sar Mountain and in the neighborhood of the lake of Akna-Litch *V. formosa* occurred in vast numbers. The third habitat of this species located in Syunik upland region (Southern Armenia), in the neighborhood of the mountains of Mets-Ishkhanasar and Ukhtasar, was identified relatively recently, in 2003 and 2006, by H. Kazaryan, and collected again in 2007 by I.Gabrielyan. In 2006-2007 Geghama and Syunik upland populations of *V. formosa* were studied within the framework of the UNEP/GEF funded Crop Wild Relatives projects, some data on the species distribution in Armenia, population size and possibilities of ex-situ conservation were given by J. Akopian and I. Gabrielyan (1).

An *in – situ* investigation of *V. formosa* was carried out in July-August 2009 with support and active participation from the Green Lane NGO (Armenia). *V. formosa* populations in two sites – Syunik upland region and Geghama highland region, which belong to different geo-morphological sub-provinces of Armenia, have been observed. In both areas *V. formosa* occurs on South facing slopes of the Upper Quaternary slag cones with slag particle size varying from several mm up to 10 cm and more. In addition, these slope areas are situated in the neighborhood of elevated lakes. In Syunik upland region, *Vavilovia* occurs near the mountain of Ukhtasar in the Mountain Range of Tskhuk, at the altitude of 3305 – 3453 m asl. From western, southern to eastern slopes, the population is spread over an area of about 800 m. The slopes of the Tskhuk Mountain Range are coated with reddish-yellow and reddish-black slag bodies (photo 1). On July 17th, the expedition reached the top area of the mountain of Ukhtasar and started searching for plants of *Vavilovia*. The first plants of *V. formosa* were found at the altitude of 3305 – 3315 m asl on the southeast slope. The seasonal development phenophase in this *Vavilovia* population was budding / inception of flowering. Then the expedition found sporadic plants with expanded flowers. *Vavilovia* plants are very beautiful, especially in blossom (photo 2).


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It must be mentioned that on the slopes observed, *Vavilovia* is accompanied by many other species of alpine plants. During the whole period of observation (14.00 to 18.00 pm), the slope stayed sunlit and sun-warmed. One had the impression that in the period between 16.30 pm and 17.30 pm the blooming process became more active, as flowering *Vavilovia* plants started occurring more often on the slope.

V. formosa is a perennial herbage plant with creeping rootstock. *V. formosa* belongs to the rubble moving mound vegetation. The population observed is narrowly adapted to the conditions of high alpine and subnival zones. At the altitude of ca. 3350-3400 m, the density of plants in the population was higher than the one identified at the foot of the slope. Probably as a result of being permanently covered by detritus, the overground offshoots develop roots by means of numerous extremely thin secondary roots that not only spread in topsoil (20-30 cm) but also go down to the soil layer underlying the detritus. It must be mentioned that plantlets with 5-7 leaves, developed rootlets and remaining seed coats, have been occurring sporadically (3-4).

In the Ukhtasar population (2), plants are from 4 up to 6 cm with 4 – 8 off-shoots, leaves and flower-bearing stems are vivid green with violet spots. Both the vegetative organs of *Vavilovia* and its flowers are in close contact with substratum, and it is interesting to measure air temperature at the level of plant. The temperature (T) on the slope, and in particular, at the surface of sun-warmed scree near the plants, was 30 – 32° C, while, on the same slope fragment, the air temperature (T) in the shade was about 16° C. After 19.00 pm the air temperature went down to 10-12° C and the surface temperature of the sun-warmed stones was (12) 15 – 16° C. It must be mentioned that there was a constant strong wind blowing at the summit of the mountain. In Syunik highland region, in the area of Ukhtasar, the average wind speed is about 7.7 meters a second, which is the highest index in the country. Winds with speed of 15 m/second and more occur very often in this region (over 85 days a year).

Cones with Upper Quaternary slag deposits are the most clearly visible in the Geghama highland, where the second site observed is located. The watershed of Hrazdan River

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and Sevan Basin is dominated by about 30 rather large cones. The Mountain of Sev Sar (3258.5 m high), situated 1 km north of Aknalitch lake is among them. In Sev Sar, the *V. formosa* population area is about 700 m long and 100 m wide. On the western, south-western and southern slopes it starts at the altitude of 3111 m asl and continues up to 3203 m. The slopes of Sev Sar are covered with incoherent slag blanket of two colors: the western part is red and the eastern part is black. *V. formosa* occurs in both areas, though on the slopes coated with reddish slag the number of plants is far larger. According to the results obtained by the expeditions organized within the framework of the UNEP/GEF funded Crop Wild Relatives projects (A.Danelyan, I. Gabrielyan, A. Melikyan) in 2007, in vicinity of Aknalitch, on the adret slopes situated on the southern bank of the lake, several hundreds of *Vavilovia* plants were found on the area of 0.5 ha. During our expedition of August, 2009, no plant was found in that area now turned into high mountain range for hundreds of animals – cattle and sheep (photo 3).

It is noteworthy that the Red Data Book of Armenia (3) identifies the *Vavilovia* population in the region of Aknalitch Lake as being extinct. These contradictory data suggest extreme instability of the population that obviously is in serious danger of extinction. Besides *Vavilovia,* in this area other rare species under protection occur - *Eunomia rotundifolia*, *Potentilla porphyrantha*, *P. seidllitziana*, *Scilla rosenii*, *etc.* After walking several tens of kilometers in search of *V. formosa,* we found the second population distributed over an area of about 4 - 4.5 ha, to the north-east of Aknalitch, on the northern slopes of a big hill at the altitude of 3045-3170 m asl, facing a small waterlogged hollow. The angle of slope was 45-55^0. In that area the plants and flowers were already touched by frost and the expedition found few flowers and dry pods as at night the air temperature was -2 -3° C. In contrast to the Ukhtasar population, the plants in the region of Aknalitch were a little more inconspicuous and mostly deep green, without spots. The number of heart-shaped leaves on each plant varies from 6 to 22. The exact size of the stems as well as the main and lateral roots have not been defined but the average length of the plants - from the flower to the root end was about 90-115 cm. There were tubercles (presumably with tuberous bacteria inside) on all the roots.

So, as a result of the summer expedition of 2009, *V. formosa* populations in two different localities of Armenia have been studied, which enriched our knowledge of its natural habitat climatic and geomorphologic conditions. Due to *Vavilovia* in-situ observations, some ecological, phenological and biomorphological peculiarities of its growth and development were revealed. The obtained data can be available for working out active measures for these rare species protection. ■

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The AEP Scientific Committee

Vth International Congress on Legume Genetics and Genomics (ICLGG)

July 2-8, 2010

Asilomar Conference Grounds, Pacific Grove, California
Registration and Information Website conferences
http://www.cevs.ucdavis.edu/conflist/
The meeting will focus on fundamental discoveries that extend our understanding of the unique traits of legumes. A special feature of the ICLGG is its mission to bring together people working on fundamental aspects of legume biology in model species, using genetic and genomic tools, with those working on applied aspects and breeding of crop and pasture species

GRAIN LEGUMES

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