Model legumes contribute to faba bean breeding

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Abstract

Faba bean is an excellent candidate crop to provide nitrogen input into temperate agricultural systems. However, its growth is hampered by several factors including environmental stresses and the presence of anti-nutritional factors. To solve these limitations, breeding programs have been initiated that were successful for monogenic traits but not so for multigenic traits. The large genome size of faba bean has slowed down breeding processes. Several other legumes have emerged as model legumes including *Medicago truncatula*, *Lotus japonicus*, *Glycine max* and *Pisum sativum*. The establishment of these models has already boosted our understanding of important processes such as the nitrogen-fixing symbiotic interaction. The high level of synteny and collinearity existing between legumes makes possible the transfer of key knowledge from model legumes to faba bean. Here we review the most recent knowledge gained from model legumes on grain quality, resistance to biotic and abiotic stresses, nitrogen-fixing symbiosis and how this knowledge can be employed for faba bean breeding.

Keywords: biotechnology, breeding, faba bean, model legumes, *Vicia faba*, *Medicago truncatula*, *Lotus japonicus*

1. Introduction

Grain legumes play a critical role in crop rotation. Thanks to the unique process of biological fixation of atmospheric N₂, grain legumes can meet two major challenges in modern agriculture: (i) reduction of fossil energy use and green house gas emission through decrease of nitrogen fertilizers, which contribute to both CO₂ and N₂O emissions; (ii) diversification of cropping systems to reduce the need for external inputs such as pesticides, to improve nutrient and water use and to reduce losses of nutrients to the environment. However, the inclusion of legumes in the cropping systems is still rather low despite their beneficial functions towards sustainable and multifunctional agroecosystems. To turn grain legumes into proper candidates for a sustainable agriculture, they should be attractive both to producers and to users (human or animal nutrition).

Faba bean is an excellent candidate crop to provide nitrogen input into temperate agricultural systems. Significant genetic variation for symbiotic parameters exists with numerous faba bean germplasm lines maintained, providing an excellent resource for plant breeders (Duc et al., 2009, this issue). Priorities for faba bean breeding are the development of resistant genotypes to biotic (Sillero et al., 2009, this issue) and abiotic constraints such as over-wintering frost (Stoddard et al., 2009a, this issue) and drought (Stoddard et al., 2009b, this issue), and free of anti-nutritional factors (Krepon et al., 2009, this issue). Understanding the bottlenecks during symbiotic signalling and the processes underlying nodule development and nitrogen assimilation are critical for the improvement of current breeding programs. Critical traits to focus on faba bean nodulation biology include high nodulation ability, tolerance to nitrate containing soils, increased symbiotic mass to increase total nitrogen fixation ability, and interaction with mycorrhizal fungi (cf. Meixner et al., 2007). Many of these traits have already been

1 incorporated into modern cultivars, but several others, many of which are controlled
2 quantitatively by multiple genes, have been more difficult to manipulate.
3 Implementation of Marker-Assisted Selection (MAS) schemes offers plant breeders a
4 mean to improve selection efficiency, reducing the time and effort required to develop
5 new cultivars. Although Quantitative Trait Loci (QTL) mapping studies have been
6 performed for almost all grain legumes, in most cases no markers are readily available
7 for QTL selection and MAS yet. The limited saturation of the genomic regions bearing
8 putative QTLs makes difficult to identify the most tightly-linked markers and to
9 determine the accurate position of QTLs (Torres et al., 2009, this issue). Effectiveness
10 of MAS might soon increase with the adoption of the new improvements in marker
11 technology together with the integration of comparative mapping and functional
12 genomics. Traditional breeding efforts will be greatly enhanced through collaborative
13 approaches using functional, comparative and structural genomics. Development of
14 novel methods to introduce genes into grain legumes through plant transformation
15 methodology promises to give plant breeders the opportunity to overcome hybridization
16 barriers and other limitations related to those traits for which little or no natural
17 resistance has been identified in addition to provide means to study gene function and
18 genome organization. Molecular genetic and genomic analyses promise the transfer of
19 technology from model legumes to faba bean, despite a generation of neglect.

21 **2. The Model Legumes**

22 In the last three decades, the study of complex biological processes in plants has
23 been facilitated by the development of the model plant, *Arabidopsis thaliana*. This
24 model has already allowed various breakthroughs in our understanding of different
25 processes such as plant development (van Hengel et al., 2004; De Smet and Jurgens,

2007) and plant response to biotic and abiotic stresses (Jones and Dangl, 2006; Swindell et al., 2007; Ma et al., 2008). However, while the study and further development of *A. thaliana* as a unique model improved greatly our understanding in some processes, its limits also became apparent. Indeed, *A. thaliana* cannot be considered as the universal model since, for instance, it is not the natural host of many pathogenic or symbiotic bacteria and fungi (Handberg and Stougaard, 1992). Thus, several other species including *Oryza sativa*, *Medicago truncatula* and *Lotus japonicus* have been more recently proposed and developed as alternative models to address specific issues of a more restricted group of plants.

M. truncatula and *L. japonicus* were initially developed as models to study the nitrogen-fixing symbiosis, restricted to the *Fabaceae*, since each species responds slightly differently to its rhizobial partner: *L. japonicus* forms determinate nodules whereas *M. truncatula* forms indeterminate nodules (Handberg and Stougaard, 1992; Rose, 2008). The fact that both are small self-fertile plants with a short growth cycle and profuse flowering and seed production makes them ideal for classical and molecular genetics since fast generation of a mapping population is easy to achieve. In addition, *M. truncatula* and *L. japonicus* are diploid legume species with eight and six chromosomes in their haploid phase respectively (Barker et al., 1990; Handberg and Stougaard, 1992). Their genomes are relatively small estimated around 500 and 470 Mb respectively (Sato et al., 2007), so only slightly higher than *A. thaliana* (125 Mb) and significantly smaller than most legume species i.e. 4,000 Mb for pea or 13,000 Mb for faba bean (Barker et al., 1990; Handberg and Stougaard, 1992). Altogether, these characteristics, along with the capabilities of these species to be transformed by *Agrobacterium tumefaciens* or *A. rhizogenes*, have made of *M. truncatula* and *L.*

1 *japonicus* valuable tools for the dissection of symbiotic interactions at the molecular
2 level and to address specific legume needs.

3 To facilitate the study of plant-microbe symbiotic interaction in legumes, different
4 tools for classical, molecular and reverse genetics, along with functional genomics were
5 developed in these two species. Several germplasm collections of both model species
6 are available that are useful to search for genetic polymorphism for particular traits.
7 Mining these collections allowed the development of several genetic maps, based on F2
8 populations, in both species using a wide array of genetic markers such as Cleaved
9 Amplified Polymorphic Sequence (CAPS), Amplified Fragment Length Polymorphism
10 (AFLP), Random Amplification of Polymorphic DNA (RAPD) and microsatellites that
11 are consolidated by the generation of Recombinant Inbred Lines (RILs) (Thoquet et al.,
12 2002; Choi et al., 2004a; Sandal et al., 2006; Wang et al., 2008). Among these maps, the
13 intra-specific maps in *M. truncatula* A17 x A20 (Ané et al., 2008) and in *L. japonicus*,
14 Gifu B129 x Mijakojima MG-20 (Wang et al., 2008), and the inter-specific map
15 between *L. japonicus* Gifu B129 and *L. filicaulis* (Sandal et al., 2006) have been used as
16 reference for map-based cloning and genome sequencing. The information obtained by
17 these genetic maps was complemented by the generation of cytogenetic maps based on
18 Fluorescence *in-situ* Hybridisation (FISH) on pachytene chromosomes, instrumental for
19 map-based cloning and comparative genomics (Kulikova et al., 2001; Pedrosa et al.,
20 2002). In addition, a genome sequencing initiative of gene-rich regions *via* the Bacterial
21 Artificial Chromosome (BAC)-by-BAC strategy and different Expressed Sequence Tag
22 (EST), sequencing programs have been initiated for *M. truncatula* and *L. japonicus*.
23 These programs have led to the creation of more than 200,000 and 100,000 ESTs,
24 available from public DNA database, for *M. truncatula* and *L. japonicus*, respectively,

1 and the sequencing of nearly 190 and 315.1 Mb of their respective genomes (Cannon et
2 al., 2006; Ané et al., 2008; Sato et al., 2008).

3 In parallel, transcriptomic and proteomic tools have also been developed in both
4 models as alternative approaches for the study of the symbiotic interaction and other
5 processes (Wienkoop and Saalbach, 2003; Colebatch et al., 2004; El Yahyaoui et al.,
6 2004; Kouchi et al., 2004; Hohnjec et al., 2005; Gallardo et al., 2007; Sanchez et al.,
7 2008). Several macro- and micro-array platforms have been developed in these species
8 initially to study the symbiotic interaction. Large-scale macro-array techniques allowed
9 the monitoring of 6,000 and 15,000 genes simultaneously in *M. truncatula* and *L.*
10 *japonicus* (El Yahyaoui et al., 2004; Kouchi et al., 2004). Targeted macro-array
11 platforms were also developed to study specific topic including a 92 defence-related
12 gene macro-array to study the *M. truncatula-Colletotrichum trifolii* interaction
13 (Torregrosa et al., 2004) or a 384 salt stress-related genes to study *M. truncatula*
14 response to salt stress (Merchan et al., 2007). Simultaneously, the first micro-array
15 platforms, developed at the University of Bielefeld (Germany) and the Max Planck
16 Institute of Molecular Plant Physiology, Golm (Germany) allowed the screening of
17 6,231 and 2,500 unique transcripts respectively (Colebatch et al., 2004; Küster et al.,
18 2004). With the progress of genome sequencing, the *M. truncatula* micro-array platform
19 was upgraded to allow the monitoring of 16,000 genes (Hohnjec et al., 2005) that have
20 also been completed with the entire *Sinorhizobium meliloti* genome to develop a dual
21 symbiotic chip (Barnett et al., 2004). In addition, Affymetrix chips with
22 bioinformatically optimized oligonucleotides are also commercially available for *M.*
23 *truncatula* and *L. japonicus* (<http://www.affymetrix.com>; Sanchez et al., 2008) and a
24 novel generation of *M. truncatula* gene chips with probe sets for 1,850 *M. sativa*
25 transcripts to facilitate transcriptomic analysis of closely related species will be soon

1 available (Ané et al., 2008). In parallel to these large-scale hybridisation techniques, a
2 method for the simultaneous monitoring of more than 700 transcription factors by
3 quantitative Polymerase Chain Reaction (PCR) has been established at the Max Planck
4 Institute of Molecular Plant Physiology, Golm (Germany) (Kakar et al., 2008). All these
5 transcriptomic platforms allowed large improvements in our understanding of legume
6 symbiotic interactions and begin to be used for other purposes including the response to
7 salinity (Sanchez et al., 2008; Gruber et al., 2009), grain filling (Gallardo et al., 2007;
8 Verdier et al., 2008), or legume-pathogen interactions (Torregrosa et al., 2004; Curto et
9 al., 2007; Ameline-Torregrosa et al., 2008; Dita et al., 2009).

10 In turn, several proteomic approaches, based on different protein separation methods
11 and identification by mass spectrometry, have been developed and applied for these
12 model species. These original approaches targeted the establishment of reference
13 protein and peptide maps in *M. truncatula* (Watson et al., 2003) and the analysis of the
14 symbiotic compartment in both *L. japonicus* and *M. truncatula* (Wienkoop and
15 Saalbach, 2003; Valot et al., 2006; Larrainzar et al., 2007; van Noorden et al., 2007).
16 More recently, the range of application of proteomic approaches has been broadened to
17 include grain filling (Gallardo et al., 2003; Gallardo et al., 2007; Repetto et al., 2008)
18 and pathogen interactions (Colditz et al., 2005; Castillejo et al., 2009). Nowadays,
19 second and third generation proteomic tools such as Differential In-Gel Electrophoresis
20 (DIGE) and isobaric Tag for Relative and Absolute Quantitation (iTRAQ) are being
21 developed in *M. truncatula* along with approaches targeting the post-translational
22 modifications including nitrosylation and phosphorylation at large scale (M.A.
23 Castillejo, personal communication).

24 Apart from these genomic tools, many reverse genetic approaches were also
25 developed in these models. To this purpose, several collections of chemical or

1 insertional mutants including T-DNA and transposon tagged lines have been created
2 (Thykjaer et al., 1995; Penmetsa and Cook, 2000; Webb et al., 2000; Kawaguchi et al.,
3 2002; Tadege et al., 2008). These collections have already been used to identify new
4 genes required for symbiosis but may also be screened for other interesting traits. The
5 improvement of PCR-based techniques for screening for mutation in gene of interest
6 allowed the development of novel approaches for efficient reverse genetic analysis.
7 Several of these novel approaches, have been or are being developed in *M. truncatula*
8 and *L. japonicus* including Targeted Induced Local Lesions in Genome (TILLING),
9 saturating *Tnt1*-insertion mutagenesis and fast-neutron mutagenesis. TILLING relies on
10 point mutagenesis with ethyl methyl sulfonate (EMS), and provides an allelic series
11 ranging from silent mutations to complete loss-of-function of the gene of interest. This
12 method was first developed for legume in *L. japonicus* (Perry et al., 2003) already
13 allowing the identification of novel symbiotic genes in this species (Heckmann et al.,
14 2006; Horst et al., 2007) and is now available in *M. truncatula* (the Grain Legumes
15 European Integrated Project (GLIP), <http://www.eugrainlegumes.org/>). In an attempt to
16 saturate the whole *M. truncatula* genome, a large collection of *Tnt1*-tagged *M.*
17 *truncatula* lines has been established along with the facilities to characterise the
18 transposon-flanking regions (Tadege et al., 2008). Fast-neutron mutagenesis detection
19 methods were also set-up for *M. truncatula* and *L. japonicus*, allowing the identification
20 of one non-nodulating mutant FNN5.2 in *L. japonicus* (GLIP,
21 <http://www.eugrainlegumes.org/>; Hoffmann et al., 2007). In addition to help identifying
22 new genes involved in plant biology, these methods can serve to identify the exact
23 function of these genes, which is a pre-requisite step before gene transfer into other
24 legume crops such as faba bean. *Tnt1* mutagenesis and related transposon or T-DNA
25 tagging which insert within the gene and Fast neutron bombardment, which generates

1 large deletions, are likely to produce gene knockouts, ideal to identify gene function but
2 the point mutants identified by TILLING may be more useful as a source of favourable
3 alleles for subsequent selection. Alternatively to these mutation-based methods, two
4 transformation-based methods, RNA interference (RNAi) and/or Virus-Induced Gene
5 Silencing (VIGS) were also established in these model legumes (Limpens et al., 2004;
6 Maeda et al., 2006).

7 Altogether all the resources developed in these two models make them ideal
8 candidates to study legume physiology and have already provided important
9 breakthroughs in our understanding of legume symbiosis. In addition, these two species
10 are also affected by most stresses limiting legume crop yield such as fungal and
11 bacterial diseases, nematodes, pests or salt stress so that the different resources
12 developed on these species provide a great advantage to improve our understanding and
13 the breeding for the specific needs of legume crops such as faba bean.

14 Alternatively of these two model legumes, several legume crops including soybean
15 and pea, have been extensively studied due to their economical importance. These two
16 crop species count on large collections of germplasms and chemical and insertional
17 mutants and are the subject of genomic sequencing initiatives. In addition, many
18 genomic tools have been and are being developed in these species including proteomic
19 and transcriptomic platforms and functional genomic approaches such as TILLING,
20 RNAi and VIGS silencing techniques (Constantin et al., 2004; Subramanian et al.,
21 2005; Zhang and Ghabrial, 2006; Cooper et al., 2008; Dalmais et al., 2008; Kaimoyo
22 and VanEtten, 2008). Thus these two legume crops can also serve as model to transfer
23 interesting traits to faba bean.

3. Synteny Between *M. truncatula*, *L. japonicus* and Grain Legumes

Depending on the degree of their evolutionary relationship, different species preserve similarities in the content, proximity (synteny) and linear order (collinearity) of genes in their genomes. This suggestion derives from the idea that the relative location of genes in a genome is an accident of history and that the decay of colinearity is simply a function of chance and divergence time. Comparative mapping and genome analysis investigate conservation and differences in gene content and order among different taxa. Originally comparative analyses of genomes were performed based on genetic maps developed with molecular markers, but the increasing availability of large-scale genome sequences (www.plantgdb.org/prj/Genome_browser.php) could make comparison more direct and extensive. In the last three decades, multiple studies, using linkage maps, revealed remarkable synteny predominantly within plant families (Paterson et al., 2000). Conserved gene order between species from distinct plant families was also identified within small-scale genomic regions (microsynteny) rather than simply among large chromosome segments (Devos et al., 1999; Stracke et al., 2004; Kevei et al., 2005; Zhu et al., 2005 and many others), although collinearity between large chromosomal blocks of markers across species separated by wider taxonomic distances has also been identified in a few cases (Lee et al., 2001; Wu et al., 2006).

Comparative genetic analysis among legumes species was launched by Vavilov's studies (1922) on series of similar heritable variations in related Papilionoid species. The first molecular proofs for the existence of macrosynteny between legumes were given by the comparison of genetic maps of economically important grain legumes. The comparison of the incomplete genetic maps of lentil (*Lens culinaris*; 2n=14) and chickpea (*Cicer arietinum*; 2n=16) with the pea linkage map revealed eight and five large syntenic blocks respectively (Weeden et al., 1992; Simon and Muehlbauer, 1997;

Ellis and Poyser, 2002). Comparison between pea and *M. sativa*, also revealed a substantial conservation in the gene order in these species. This comparison allows to identify the genetic rearrangements that occurred and account for their chromosome number difference (8 for alfalfa and 7 for pea; Kaló et al., 2004), which also indicated that the 10-fold difference in their genome size is not the result of large scale pea genome multiplication. Completing the *M. truncatula* genetic map with *M. sativa* gene-based genetic markers (Kaló et al., 2000) identified the homologous linkage groups and showed an almost complete colinearity between these two related species except for the rDNA chromosomal localization (Choi et al., 2004a). For the Phaseolid legumes, a high level of collinearity and synteny was detected as shown between the genome of several *Vigna* species (Menancio-hautea et al., 1993; Kaga et al., 2000) and between *V. radiata* (mungbean) and the phaseolid legumes *Dolichos lablab* (Humphry et al., 2002) and common bean (Boutin et al., 1995). Comparison of genetic maps between soybean and common bean revealed only short conserved linkage blocks in common bean that often corresponded to nearly entire linkage groups or large contiguous blocks in soybean (Boutin et al., 1995; Lee et al., 2001).

The genomic resources developed for *M. truncatula*, *L. japonicus*, soybean and common bean boosted comparative genomic analyses between model legumes and legume crops by allowing more comprehensive macrosynteny analyses such as those reported by Choi et al. (2004b) and Zhu et al. (2005). Cross-species gene specific markers were used to identify homologous genome segments among eight legume species (*M. truncatula*, alfalfa, *L. japonicus*, pea, chickpea, soybean, mungbean and common bean). Using the *M. truncatula* genetic map as a reference genome, the eight legume genomes were aligned and a simplified consensus map was created. The degree of collinearity between legumes reflected their phylogenetic relationship. The large

amount of genomic sequences generated between the two model legumes *M. truncatula* and *L. japonicus* allows a more comprehensive in-clade comparison showing several macrosyntenic regions and significant microsyntenic regions conserving many genes in the same order and orientation (Fig. 1; Choi et al., 2004b; Cannon et al., 2006). The comparison of the microstructure of the *MtDMI2(NORK)/LjSYMRK* region also revealed a nearly complete conservation in gene content and order between the two species within a 276 kb long *M. truncatula* chromosomal segment (Kevei et al., 2005; Zhu et al., 2005). The comparative mapping between *M. truncatula* and soybean identified eleven colinear blocks with a high degree of microsynteny (Choi et al., 2004b; Mudge et al., 2005). Similarly, segments of eight linkage groups of common bean (2n=22) exhibited conservation with *M. truncatula* linkage groups (Choi et al., 2004a; 2004b).

A similar approach – generating intron-targeted gene-based anchor markers for legume species and using *M. truncatula* as a reference genome for comparative mapping - was applied in the comparative mapping program of GLIP (<http://www.eugrainlegumes.org/>) to analyse macrosyntenic relationship between pea, chickpea, faba bean, common bean, lupin and lentil. The alignment of the legume genetic maps is currently underway and preliminary data show that high level of macrosynteny exists between the genomes of *M. truncatula*, lentil (Phan et al., 2006), faba bean and chickpea (Gutierrez et al., 2008b; P. Winter personal communication). These analyses also indicated a complex syntenic pattern between *M. truncatula* and lupin for which individual *M. truncatula* chromosomes were syntenic to at least two lupin linkage groups accounting to the higher lupin chromosome number (Nelson et al., 2006; Phan et al., 2006; Phan et al., 2007). In parallel, syntenic analysis between *M.*

1 *truncatula*, *L. japonicus* and peanut (*Arachis hypogea*), a more distant grain legume,
2 revealed significant macrosynteny between these species (Hougaard et al., 2008).

3 These recent comparative genomic studies have mainly used *M. truncatula* as a
4 reference genome and revealed that colinearity exists between legume species to
5 different extents depending on their phylogenetic distance. In order to support
6 comparative legume biology the Legume Information System (LIS) was developed
7 (Gonzales et al., 2005) few years ago, which integrates genetic and physical map data
8 and enables macrosynteny analyses to be carried out between legume species *in silico*.

9 Both *M. truncatula* and faba bean are cool season legumes falling into two separate
10 tribes; faba bean (2n=12) belongs to the *Viciae* and *M. truncatula* is a species in
11 *Trifoliae* tribe (Zhu et al., 2005). The genome size of faba bean is about 25-fold larger
12 than the genome of *M. truncatula*, which restrained the development of faba bean
13 genomics. A composite genetic map of *V. faba* has been constructed (Román et al.,
14 2004) and, in the frame of GLIP, intron-targeted gene-based markers have been
15 developed and tested for faba bean. The comparative mapping between faba bean and
16 *M. truncatula* is in progress (Gutierrez et al., 2008a). Based on the phylogenetic
17 distance between the two species, large scale genome conservation is expected and the
18 identification of chromosome rearrangements responsible for different chromosome
19 number is likely, as it has been detected between *M. truncatula* and pea (Choi et al.,
20 2004b; Kaló et al., 2004). The expected high level of conservation in gene order
21 between faba bean and *M. truncatula* implies that *M. truncatula* genomic tools will
22 facilitate breeding and research of faba bean.

23 24 25 **4. Strategy for Faba Bean Improvement**

1 A major aim for any crop breeding program is the development of good quality lines
2 with an adequate resistance/tolerance to yield-reducing stresses. The use of model
3 legumes for comparative functional genomics may bring some new perspectives and
4 enhance faba bean breeding efforts. In this way, identification of QTLs and/or candidate
5 genes involved in stress tolerance and/or quality may be used to produce transgenic
6 lines and/or these traits can be applied to breeding programs (e.g., MAS).

7 Little is known about the functional correspondence of model legume genes and
8 their putative faba bean orthologues. Notwithstanding the lack of information,
9 predictions can be made based on the sequence similarities between the relatively few
10 *M. truncatula* and faba bean gene pairs that are available and the high conservation and
11 synteny existing between legume genomes. Whereas for highly conserved genes,
12 favourable mutations observed in model legumes are likely to correspond to favourable
13 alleles in faba bean, for less conserved genes (i.e. many transcription factors), the
14 relation is less reliable. Possible complications include 1) differences in gene copy
15 number, 2) differences in transcript or protein abundance, 3) differences in specific
16 activity. Therefore, the information obtained in model legumes can be used as a guide to
17 narrow down candidate genes, but proof can only come from functional studies,
18 preferably in the homologous system.

19 Once a series of candidate genes to improve a particular trait has been identified
20 in one of the model legumes, a number of options are possible for exploiting this
21 information in legume crops and particularly in faba bean breeding. The involved steps
22 are: 1) confirmation of candidate gene function either directly in faba bean or indirectly
23 in any of the model legumes, 2) identification of favourable alleles for selection, 3)
24 variety improvement by MAS or by transformation of an elite line.

Several approaches have been developed to confirm candidate gene function at the biochemical and physiological level. Originally, functional analysis of proteins was performed through two main techniques, protein over-expression and monitoring of promoter activity. Over-expression of a candidate gene is obtained by transferring the coding region of the gene under control of a strong promoter such as the *CaMV 35S* into the plant and function is assigned by scoring the phenotype of the resulting transformed line (Shimoda et al., 2008; Vernié et al., 2008). Promoter activity analysis is performed by linking the promoter sequence to reporter gene such as the beta-glucuronidase (GUS) or the green fluorescent protein (GFP) to allow analysis of tissue-specific expression (Hayashi et al., 2008). Both procedures require gene transfer that is difficult in large seeded legumes. This limitation can often be short-cut by hairy root transformation that is easier to achieve but only allows analysis of gene constructs in root tissue. Albeit with low efficiency, protocols for both *A. tumefaciens* and *A. rhizogenes* transformation have been established for faba bean and can be used for gene functional analysis in this species (Böttinger et al., 2001; Vieweg et al., 2004). Alternatively, the functional analysis could be performed in the model legumes *M. truncatula*, *L. japonicus* or soybean for which the transformation protocols are more efficient and rapid (Lombardi et al., 2003; Crane et al., 2006; Kereszt et al., 2007; Rech et al., 2008).

In these model legumes, gene function can also be removed by modern molecular genetic techniques including RNAi (Wesley et al., 2001), VIGS (e.g. Kachroo et al., 2008) and even TILLING (Colbert et al., 2001). The TILLING approach is also available for *G. max* (Cooper et al., 2008) and *P. sativum* (Dalmais et al., 2008) but not yet for faba bean, the difficulty being generation and maintenance of a large perfectly homozygous population for mutagenesis.

Once the function of a candidate gene has been validated, identification of favourable alleles has to be performed. Defining patterns of synteny and collinearity between species by comparative genomic studies (cf. section 3) helps the identification of orthologous genes in genetically recalcitrant species as compared to model systems. Once a gene behind a given phenotype has been identified by a map-based cloning approach and validated, the orthologous gene in the other species can be isolated based on similar map position. There are several examples for this fruitful approach among the legume species where genes involved in symbiotic interactions have been identified (e.g. pea mutants *sym19* - *DMI2/NORK*, *sym2* - *LYK3*, *sym7* - *NSP2*; Endre et al., 2002; Limpens et al., 2003; Kaló et al., 2005). The syntenic map position of the dwarf phenotype in diploid alfalfa (*Msdwf1*) and pea (*le*) and the genomic resources in *M. truncatula* enabled the identification of a gene encoding a gibberellin 3- β -hydroxylase (GA3ox) required for normal growth habit in diploid alfalfa (Dalmadi et al., 2008). These examples clearly demonstrate the two-way utility and application of molecular markers and the identified orthologous regions between the genomes of reference and crop legumes. The tools developed in model species can facilitate the identification of agronomically important genes (QTLs, genes involved in nutrient quality and quantity, biotic and abiotic stresses, etc.) and marker-assisted breeding programs in target organisms while the accumulated biological knowledge in crop species can contribute the understanding of biological processes. Alternatively, selection of favourable alleles of the gene of interest can be found using the EcoTILLING approach that allows the detection of allelic variants of a candidate gene in natural populations for their subsequent phenotyping for the trait in question. Finally, the favourable allele can be transferred to elite faba bean cultivars by MAS or genetic transformation.

5. Application of Model Legumes to Faba Bean Improvement

5.1. Breeding for quality

5.1.1. Model legumes and quality traits

M. truncatula seed biology is essentially very similar to that of the major temperate crop legumes, pea and faba bean, but differs in that the major carbon reserves are lipids, rather than starch, which is present only in trace amounts in the mature seed. The *M. truncatula* seed also contains about 10% of endosperm material at maturity, unlike pea or faba bean in which this layer is reabsorbed during development. As *M. truncatula* was not bred for grain consumption, its seeds are also relatively small with a relatively high proportion of cell wall material and a low harvest index. Proteins represent the major class of storage compounds in *M. truncatula* seeds, followed by lipids, with only trace quantities of starch (Duc, 2004; Djemel et al., 2005). Whereas proteins and oils are coordinately synthesized during seed filling, the non-starch carbohydrate fraction (mainly trachyose) accumulates only at the end of seed maturation, when seeds are acquiring desiccation tolerance. Fatty acid and sugar compositions are similar to those of pea and other grain legumes. Thus, with certain caveats, the *M. truncatula* seed is a good model for identifying genes important in regulating seed composition in grain legumes.

5.1.2. Identification of grain quality characters

The availability of a comprehensive EST database has allowed a large-scale identification of genes putatively encoding *M. truncatula* seed proteins that have been subsequently confirmed by seed protein separation and Matrix-Assisted Laser Desorption/Ionization – Time-of-Flight (MALDI-TOF) analysis, some of which are candidate genes for quality traits (Watson et al., 2003; Gallardo et al., 2003). The major

1 *M. truncatula* storage proteins are the 7S (vicilin and convicilin-type) and 11S
2 (legumin-type) globulins, with similar amino acid compositions to those of other grain
3 legumes, notably being poor in sulphur-containing amino acids (Gallardo et al., 2003).
4 The storage proteins accumulate sequentially during seed filling, the vicilins at 14 days
5 after pollination (DAP) followed by the legumins (16 DAP) with the convicilins
6 accumulating last (18 DAP).

7 Among the proteins identified at different developmental stages, several
8 enzymes and other proteins playing key roles in the seed were detected. For example,
9 cell division-associated proteins were expressed during the differentiation phase
10 preceding seed filling. Storage protein accumulation was accompanied by the
11 expression of putative chaperonins and protein disulphide isomerases. During this
12 phase, two PV100-like polypeptides also accumulate (Yamada et al., 1999) giving rise
13 to a trypsin inhibitor and a cytotoxin-related peptide upon processing, which are
14 important targets for breeding as their elimination could improve nutritional quality of
15 legume seeds.

16 Starch accumulates only transiently in *M. truncatula* seeds, in contrast to the
17 starch-rich pulse pea and faba bean, but similarly to the situation in soybean, starch
18 remobilisation is the contributing carbon source for oil biosynthesis (Duc, 2004).
19 Certain starch-remobilisation enzymes (starch synthase, sucrose synthase and triose
20 phosphate isomerase) were transiently expressed 16-24 DAP, concomitantly with
21 proteins involved in photosynthesis, supporting the hypothesis that photosynthesis in the
22 embryo provides energy for lipid biosynthesis, which may also recycle fixed CO₂
23 (Gallardo et al., 2003).

24 Seed development involves the interplay of several tissues; the developing
25 embryo is surrounded by the endosperm, and the two organs are embedded in the

maternal integument. The role played by the embryo-surrounding tissues in legume seed reserve accumulation has been investigated genetically for pea and soybean (Lemontey et al., 2000), indicating significant maternal effect early in seed filling. To study these interactions in more detail and get access to the genes involved, gene expression in these tissues has been analysed at the proteome and transcriptome levels (Gallardo et al., 2007). A general observation is that the pattern of proteins and transcripts expressed in the embryo, endosperm and integument is very specific for each cell type, with little overlap. One of the major findings was an extensive compartmentalization of amino acid metabolism between seed tissue components that may favour storage product accumulation. Of particular interest is the compartmentalization of enzymes of sulphur amino acid biosynthesis, observed for both methionine and cysteine, as these are limiting in grain legumes.

The dependence of the embryo's nutrition on the maternal tissue was also demonstrated directly by an *in vitro* culture experiment in which embryo development on nitrogen nutrient-free medium with and without the surrounding tissue was compared (Gallardo et al., 2006). Embryos grown without nitrogen source aborted, whereas embryos grown in presence of the surrounding endosperm and integument developed normally and accumulated reserve proteins, presumably due to nitrogen remobilisation from maternal tissues. This remobilisation of a temporary nitrogen store requires proteolysis, and candidate proteases with appropriate expression kinetics have been identified in endosperm and seed coat tissues (Gallardo et al., 2007).

Seed developmental programme is under tight transcriptional control, and there is evidence from other plant systems that an important class of loci regulating seed composition corresponds to transcription factors (Le et al., 2007). To identify transcription factors (TFs) expressed in developing *M. truncatula* seeds, expression of

more than 700 TF sequences was monitored by quantitative real-time PCR throughout seed development (Verdier et al., 2008). By clustering the data of TF expression with storage protein expression profiles previously obtained, candidate factors potentially controlling the major storage protein groups were identified. In parallel, a biochemical approach analysing the nuclear proteome led to the identification of several putative regulatory proteins (Repetto et al., 2008), the functions of which remain to be determined by reverse genetics. Identified genes and proteins from all these studies may serve as quality markers potentially transferable to crop legumes for breeding once their involvement in seed quality is determined and polymorphism for these traits are found.

A survey of natural variations in seed protein complements carried out on 50 diverse *Medicago truncatula* ecotypes or cultivars indicated a high degree of polymorphism in protein composition and a large variation in protein content (33-46%) (Le Signor et al., 2005). Clustering of genotypes according to similarity in one-dimensional protein profiles allowed structuring into classes that corresponded to 4 species groups within the *M. truncatula* species complex. This classification has allowed the selection of RIL parents for maximizing variation in protein content and type in the populations to be examined, and mapping of QTLs is in progress. In a new project, expressional gene candidates, selected from the cited studies, are being mapped directly on the genetical-physical *M. truncatula* map for comparison with the positions of mapped traits. So far, around 50% of the gene candidates have been mapped, giving a total of around 750 loci, including transcription factors, nutrient transporters and other seed-specific enzymes of metabolism (A. Bordat, personal communication). A survey of QTLs for traits affecting vegetative plant development and seed yield and content in pea (Burstin et al., 2007) revealed the importance of genes determining plant architecture in controlling seed yield and protein content. It would appear likely that the homologous

1 loci in faba bean have the same properties, and therefore these should form part of
2 selection schemes.

3 Apart from selection for seed size and seed number per pod, quality breeding in
4 faba bean has to date concentrated on the reduction/elimination of the anti-nutritional
5 factors condensed tannins, vicine and convicine, responsible for favism, a severe
6 digestive disorder in susceptible individuals, which reduce nutritional value of faba
7 bean (Gutierrez et al., 2006; Gutierrez et al., 2007; Gutierrez et al., 2008a). CAP
8 markers have been obtained for the convicine locus *v-c*, and SCAR markers for the
9 tannin loci *zt-1* and *zt-2* for use in introgression of favourable alleles in breeding
10 selection. Linked molecular markers such as these may be subject to recombination with
11 the trait of interest. With the sequence data available from *M. truncatula*, there should
12 be the possibility of identifying genes encoding the responsible enzymes, and thus of
13 obtaining non-recombining and hence more reliable SNP markers within the gene itself.
14 The development of inbred lines, perhaps based on the closed flower mutation (Poulsen,
15 1977), would facilitate genetic analyses.

16 17 5.2 Breeding for resistance to biotic stresses

18 Grain legume and in particular faba bean are challenged by many pathogens and
19 pest including bacterial, virus and fungal diseases as well as infection by nematodes and
20 some parasitic plants which strongly affect crop yield worldwide (see Pérez-de-Luque et
21 al., 2009, this issue; Sillero et al., 2009, this issue; Stoddard et al., 2009c, this issue).
22 Genetic resistance is considered the most desirable control method since it is more cost
23 effective and environment-friendly than the use of chemicals. Thus, many resistance
24 sources (Sillero et al., 2009, this issue) and their associated QTLs have been found in
25 different grain legumes including faba bean (Torres et al., 2009, this issue). However,

1 the long genetic distance existing in most cases between the identified genetic markers
2 and the resistance QTLs, the common lack of codominant markers and the general lack
3 of knowledge on resistance mechanisms in legumes limit greatly the use of genetic
4 markers to confer resistance to grain legumes. The model legumes *M. truncatula* and *L.*
5 *japonicus* are affected by many of the pathogens and pest limiting faba bean yield.
6 Thus, they offer a great opportunity to improve the knowledge in resistance mechanisms
7 against faba bean pathogens and identify effective resistance genes against them.

8 Fungal and oomycete pathogens are the most diverse group of pathogens and
9 cause the most dramatic damages on legume yield worldwide. Annual *Medicago* and *M.*
10 *truncatula* in particular are strongly affected by a wide range of foliar and soil-borne
11 necrotrophic fungi which makes of *M. truncatula* a promising model to study the plant-
12 necrotrophic fungi interaction (reviewed in Tivoli et al., 2006). Several studies revealed
13 that *M. truncatula* is a potential host not only of necrotrophic fungi but also of several
14 biotrophic fungal and oomycete pathogens including *Aphanomyces euteiches* (Moussart
15 et al., 2007), *Colletotrichum trifolii* (O'Neill and Bauchan, 2000), *Erysiphe pisi* (Prats et
16 al., 2007), *Fusarium* spp. (Barbetti and Allen, 2005), *Leptosphaerulina trifolii* (Barbetti,
17 2007), *Mycosphaerella pinodes* (Moussart et al., 2007), *Phoma medicaginis* (O'Neill et
18 al., 2003; Ellwood et al., 2006; Barbetti, 2007), *Peronospora trifoliorum* (Yaege and
19 Stuteville, 2000), *Uromyces striatus* (Rubiales and Moral, 2004). In most cases,
20 screening of germplasm collections of *M. truncatula* allowed identification of a wide
21 range of differential responses to the pathogen from highly susceptible to resistant
22 (Moussart et al., 2007; Prats et al., 2007). This serves as bases for the characterisation of
23 underlying resistance mechanisms at the cellular and molecular levels as well as for the
24 identification of defence genes and QTLs responsible for resistance.

1 *M. truncatula* resistance against *P. medicaginis* and *C. trifolii* was found to be
2 controlled by single major genes, named *rmpm1* and *RCT1* respectively. These major
3 genes localised at the top of the linkage group 4 in a region containing a cluster of
4 several nucleotide binding site (NBS) – leucine rich repeat (LRR) proteins that are often
5 plant resistance (R) genes (Torregrosa et al., 2004; Yang et al., 2007; Kamphuis et al.,
6 2008). Interestingly, the *RCT1* gene of *M. truncatula* has been successfully transferred
7 to alfalfa to confer anthracnose resistance (Yang et al., 2008). Resistance to *A.*
8 *euteiches*, *M. pinodes*, *U. striatus*, *P. trifoliorum* or *E. pisi* appears to be controlled by
9 different defence mechanisms. For instance, screening of an USDA collection of *M.*
10 *truncatula* germplasms for *E. pisi* resistance indicated that resistance to powdery
11 mildew was controlled by papilla formation, by early hypersensitive response and also
12 by post-haustorial mechanisms (Prats et al., 2007). Mapping of the QTLs controlling
13 resistance to these fungal pathogens in *M. truncatula* is now underway (D. Rubiales,
14 personal communication).

15 In parallel, the transcriptomic and proteomic approaches developed for this
16 model legume are being used to understand the molecular components and to identify
17 candidate genes involved in *M. truncatula* defence against these fungal pathogens. For
18 instance, a Subtractive Suppression Hybridisation (SSH) library indicated that
19 Pathogen-Related (PR)10 proteins and proteins associated with abscisic acid signalling
20 play important roles in the *M. truncatula* resistance against *A. euteiches* (Nyamsuren et
21 al., 2003). The crucial role of PR10 in *A. euteiches* resistance was confirmed by
22 comparative proteomic and gene silencing approaches, which indicated that PR10
23 silencing led to increased resistance by antagonist induction of other PR genes (Colditz
24 et al., 2004; Colditz et al., 2007). Comparison of the proteomic profile of several *M.*
25 *truncatula* lines with varying levels of resistance also identified other proteins

1 potentially involved in *A. euteiches* resistance such as proteasome alpha subunits
2 (Colditz et al., 2005). Comparison of expression profiles of 92 defence-related genes by
3 macroarray between a resistant and a susceptible line of *M. truncatula* at key steps of *C.*
4 *trifolii* infection also highlighted the important role of PR proteins and in particular
5 PR10 in resistance. As expected, this analysis indicated that a large proportion of genes
6 present on the macroarray membrane were upregulated in the resistant *M. truncatula*
7 line while these genes were mainly downregulated in the susceptible line. Microarray
8 analysis of several *M. truncatula* genotypes with different defence mechanisms against
9 *E. pisi* allowed the identification of a set of genes involved in these defence mechanisms
10 (Curto et al., 2007; Foster-Hartnett et al., 2007). Post-genomic approaches are also
11 being applied to tackle other fungal diseases such as *M. pinodes* (Fondevilla et al.,
12 2008) and *U. striatus* (Madrid et al., 2008).

13 Legumes are also affected by bacterial pathogens. In particular, *M. truncatula*
14 can be infected by the causing agent of the bacterial wilt disease, *Ralstonia*
15 *solanacearum*, which also infects a large number of crops including tomato, potato and
16 cultivated legumes such as faba bean. A recent study showed that one *M. truncatula*
17 line, F83005.5, susceptible to *C. trifoliorum* and *P. medicaginis*, was resistant to most
18 *R. solanacearum* isolates (Vailleau et al., 2007). A major QTL was mapped on
19 chromosome 5 and two minor ones on chromosome 3 and 7 that may be helpful for
20 MAS (Vailleau et al., 2007).

21 Nematodes are also an important cause of yield losses in legumes. Interestingly,
22 *M. truncatula* and *L. japonicus* have been shown susceptible to most nematodes
23 affecting legumes. For instance, *M. truncatula* can be colonised by the stem nematode
24 *Ditylenchus dipsaci*, causing disease in many legumes such as alfalfa, pea and faba bean
25 (Plowright et al., 2002; Moussart et al., 2007). By screening a *M. truncatula* germplasm

1 collection, Moussart et al. (2007) identified several resistant and susceptible *M.*
2 *truncatula* lines that will surely allow a better understanding of stem nematode-legume
3 interaction. *L. japonicus* and *M. truncatula* are also infected by different root-knot and
4 cyst nematodes belonging to the *Meloidogyne* and *Heterodera* genera. Interestingly,
5 Weerasinghe et al. (2005) showed, in *L. japonicus*, that root-knot nematode and
6 rhizobium interactions may share common pathways. Indeed they found that *L.*
7 *japonicus* mutants deficient for nitrogen-fixing symbiosis establishment were more
8 resistant to *Meloidogyne incognita* than the wild type while a hypernodulating mutant
9 was infected to a higher extent by the nematode (Weerasinghe et al., 2005). On the other
10 hand, screening of *L. japonicus* ecotypes revealed differential infection responses
11 according to the ecotype ranging from susceptible to resistant to this nematode. Such
12 genetic diversity is being used to map and identify genes and/or QTLs involved in root-
13 knot nematode resistance (Poch et al., 2007).

14 Although less studied, legumes are also under the thread of viruses. Despite the
15 damage they cause, very little is known about virus resistance mechanisms and nearly
16 no studies have aimed at the characterisation of virus resistance in the two model
17 legumes *M. truncatula* and *L. japonicus*. The only report published to date indicated
18 that *L. japonicus* could be infected by *Arabis mosaic virus* and *Tobacco ringspot virus*
19 while it was resistant to most legume infecting viruses (Schumpp et al., 2007). Due to
20 their economic importance, virus diseases have been more studied in soybean, which
21 thanks to its relatively small genome and the development of genomic tools begins to be
22 considered as the third model legume (Maroof et al., 2008b). In this species, several
23 resistance genes to the soybean mosaic virus have been identified and pyramided in a
24 single cultivar (Maroof et al., 2008a). In parallel, Babu et al. (2008) found that during
25 the susceptible interaction, the defence reaction was only activated at the latest stages of

the interaction, which may be critical for the systemic infection of the virus. Independently, several transgenic approaches have been undertaken leading to increased resistance against several viruses including the soybean mosaic virus (Furutani et al., 2007) and the soybean dwarf virus (Tougou et al., 2007).

In semi-arid regions worldwide, including Southern and Eastern Europe, North and East Africa and the Middle East, parasitic plants of the *Orobanche* spp. including *O. crenata*, *O. aegyptiaca* and *O. foetida* drastically decrease legume yield. *M. truncatula* has been recently proposed as a model to study the interaction *Orobanche* spp. – legumes (Rodriguez-Conde et al., 2004; Lozano-Baena et al., 2007; Fernández-Aparicio et al., 2008). *L. japonicus* can be infected by *O. aegyptiaca*, but shows incompatible interaction against *O. minor*, *Striga hermonthica* or *S. gesnerioides* (Kubo et al., 2009). Even when *L. japonicus* is not infected by *O. minor*, its root exudates have strong stimulatory activity of *O. minor*, as well as of *O. crenata*, *O. densiflora*, *O. aegyptiaca* and *O. ramosa* seeds (Fernández-Aparicio et al., 2009).

To improve our understanding of the *M. truncatula*-*O. crenata* interaction, a SSH library has been created, allowing the identification of around 300 candidate genes for *O. crenata* defence (Dié et al., 2007). In addition, a microarray analysis of the *M. truncatula* genes regulated in response to *O. crenata* was recently performed on the M16kOLI1 microarray platform (M.A. Dita, unpublished). A comparison of two-dimension proteomic profile of two *M. truncatula* genotypes varying in their level of resistance against *O. crenata* was also performed (Castillejo et al., 2008). Preliminary analysis of the comparison of the transcriptome of two *M. truncatula* genotypes with different resistance mechanisms indicated significant changes in the steady-state level of many transcripts belonging to several functional categories, including pathogen-induced genes, such as PR genes, hormone-associated genes and transcription factors. These

analyses also revealed the activation of both the salicylic acid and jasmonate defence-pathways (M.A. Dita, unpublished). These preliminary results support the previously established results and should prove useful to identify potential candidate genes for crop improvement. These candidate genes should be validated through functional analysis. Validated candidates may then be used for genetic improvement of crop either directly through genetic transformation or indirectly by MAS.

5.3. Breeding for resistance to abiotic stresses

Global climate change predictions suggest new scenarios with larger arid areas and extreme climatologic events. Thus, it is essential to understand how plants respond to different abiotic stresses in order to improve crop performance. This difficult task can only be achieved by integrating conventional breeding and biotechnological approaches (Chaves et al., 2003). However, most legume crops are not easily amenable for molecular and genetic studies. To circumvent this limitation knowledge gained on the two model legumes *M. truncatula* and *L. japonicus* may be further used to understand the responses to abiotic stresses in other legumes such as faba bean.

Among the numerous environmental constrains affecting crop yield, drought is considered the most limiting factor with important economic consequences (Jones, 2004). *M. truncatula* is quite a drought-tolerant plant species compared to grain legumes such as pea (González et al., 1998; Gálvez et al., 2005) or soybean (González et al., 1995). Based on physiological and biochemical studies, *M. truncatula* responses to drought appear to be similar to those described in alfalfa (Rubio et al., 2002; Naya et al., 2007). The relative drought tolerance of *M. truncatula* has been shown in a recent study, where moderate water deficit had only a slight significant effect on plant biomass, presenting some differences among cultivars/ecotypes (Limami et al., 2006).

1 Nunes et al. (2008) have further corroborated this relative tolerance, showing that under
2 mild drought conditions *M. truncatula* plants were able to avoid leaf dehydration and
3 under severe drought stress plants maintained significantly high net CO₂ fixation rates.

4 Particular emphasis has been laid on the regulation of symbiotic nitrogen fixation
5 (NF) under drought stress in nodulated legumes. In contrast to earlier studies in soybean
6 (González et al., 1995) and pea (González et al., 1998; Gálvez et al., 2005), analysis in
7 *M. truncatula* suggests that the drought-induced downregulation of sucrose synthase is
8 not the main responsible for the inhibition of NF (R. Ladrera, E.M. González, C.
9 Arrese-Igor, unpublished), similarly to observations in *M. sativa* (Naya et al., 2007).
10 Additionally, the response to drought at the nodule level has been recently analysed
11 under a proteomic perspective (Larrainzar et al., 2007), where new marker enzymes
12 such as plant methionine synthase and bacteroid serine hydroxymethyltransferase were
13 identified. Regarding *L. japonicus*, Díaz et al. (2005) reported an accumulation of
14 proline and oxidative damage in leaves upon different water deprivation treatments.
15 Although the first studies analysing the response of this legume to water deficit have
16 started to emerge, most of the published work so far is based on other *Lotus* spp.
17 (Olsson et al., 1996; Carter et al., 1997; Borsani et al., 1999; 2001; Banon et al., 2004).

18 Plant responses to salt stress have been extensively analysed (reviewed in
19 Hasegawa et al., 2000; Yamaguchi and Blumwald, 2005), with an especial emphasis on
20 the role played by different osmolytes in homeostasis maintenance. Some compounds
21 such as proline-betaine, trehalose or trigonelline, a pyridine betaine, have been reported
22 to play a role in the response to salt stress of different legumes (Tramontano and Jouve,
23 1997; Trinchant et al., 2004; López et al., 2008). Furthermore, proline accumulation has
24 been shown to enhance NF during salt stress in *M. truncatula* (Armengaud et al., 2004;
25 Verdoy et al., 2006). In a recent functional analysis, Sanchez et al. (2008) reported a

1 general increase in the steady-state level of many amino acids, sugars and polyols, with
2 a concurrent decrease in most organic acids in response to gradual salt stress in *L.*
3 *japonicus* leaves. On the other hand, molecular approaches have been applied to
4 examine the response of *M. truncatula* and *M. sativa* under salinity leading to the
5 identification of several transcription factors related to the plant root response to salt
6 stress (Merchan et al., 2003; de Lorenzo et al., 2007; Merchan et al., 2007).

7 The effect of low temperatures on plants has also received considerable attention.
8 Unfortunately, little is know about the response of legumes to this type of stress, as
9 most of the published reports are based on model plants such as *A. thaliana*. Plant cold
10 acclimation is a complex process, which involves the specific expression of cold-
11 induced genes to stabilize membranes against freeze-induced injury. This group
12 includes genes encoding late embryogenesis-abundant proteins, enzymes required for
13 osmolyte biosynthesis, antifreeze proteins, chaperones and detoxification enzymes,
14 under the control of several cold-induced transcription factors (Thomashow, 1999;
15 Heino and Palva, 2003). Based on the information available, it appears that *M.*
16 *truncatula* exhibit a poor freezing tolerance, when compared to other annual legumes
17 (Brandsaeter et al., 2002). This might be due to an ineffective cold acclimation process
18 and low starch reserves in this species (Hekneby et al., 2006). Interestingly, the *M.*
19 *truncatula* ZFP1 gene, encoding a root-enhanced zinc finger protein with high similarity
20 to a soybean cold-inducible protein, is not regulated by low temperature, suggesting a
21 different physiological function of this protein in both legume species (Xu and Ma,
22 2004). Some promising results for low temperature legume breeding have been obtained
23 by transgenic expression of an iron-superoxide dismutase in alfalfa, resulting in an
24 enhanced winter survival (McKersie et al., 1993; 2000)

1 Flooding is another environmental stress that negatively influences germination,
2 seedling establishment and plant development, as it causes a limitation in the flux of
3 oxygen to support plant respiration (Bailey-Serres and Voesenek, 2008). Besides the
4 activation of alcohol and lactic fermentative pathways, flooding stress on *M. truncatula*
5 seedlings induces activity of mitochondrial alanine aminotransferase and glutamate
6 dehydrogenase which may contribute to the maintenance of the redox balance during
7 fermentative growth (Ricoult et al., 2005; 2006). The involvement of non-symbiotic
8 hemoglobins in flooding stress adaptation has been shown in *L. japonicus* (Shimoda et
9 al., 2005), and soybean (Lee et al., 2004), whereas promoter analysis carried out in faba
10 bean suggested that symbiotic leghemoglobins were not induced upon hypoxia (Vieweg
11 et al., 2004).

12 In the context of GLIP European project, abiotic stress tolerance has been focused to
13 species such as *M. truncatula*, pea and chickpea (Gálvez et al., 2005; de Lorenzo et al.,
14 2007; Larrainzar et al., 2007; Merchan et al., 2007; Marino et al., 2008) leading to
15 identification of factors potentially involved in abiotic stress adaptation and tolerance.
16 The involvement of some genes in abiotic stress response has been already analysed in
17 different legumes. For instance, alfalfa over-expressing chloroplastic MnSOD showed
18 lower cold-induced membrane injuries (McKersie et al., 1996), although these
19 transgenic lines did not present better tolerance to drought stress (Rubio et al., 2002).
20 The transcriptional regulator, *Alfin1*, over-expressed in alfalfa was shown to regulate
21 endogenous NaCl-inducible gene expression, resulting in salinity tolerance (Winicov
22 and Bastola, 1999). Similarly, a drought-responsive AP2-type transcription factor
23 induced several wax-related genes resulting in increased drought tolerance when over-
24 expressed in alfalfa (Aharoni et al., 2004; Zhang et al., 2005). In addition, the stress-

inducible expression of AtDREB1A increases transpiration efficiency in peanut under water-limiting conditions (Bhatnagar-Mathur et al., 2007).

5.4. Breeding for nitrogen fixation

5.4.1. Induction of legume root nodules

Nodulation is initiated by plant roots exuding flavonoid molecules into the soil (Ferguson and Mathesius, 2003). This attracts rhizobia to the roots and concomitantly stimulates them to synthesize a lipochito-oligosaccharide signaling molecule called Nod Factor (NF) (Caetano-Anollés and Gresshoff, 1991; Stacey et al., 2006; Oldroyd, 2007). Using the model species *L. japonicus* and *M. truncatula*, and a predominantly mutant-based approach, many of the genes required for nodule development have now been elucidated (Stacey et al., 2006; see Fig. 2A). This includes genes encoding transmembrane LysM-type receptor kinases believed to be required for NF perception: *LjNFR1* and *LjNFR5* in *L. japonicus*, and *MtNFP*, *MtLYK3* and *LYK4* in *M. truncatula* (Ben Amor et al., 2003; Limpens et al., 2003; Madsen et al., 2003; Radutoiu et al., 2003; Arrighi et al., 2006). Subsequent to perception, NF signaling continues through a NBS-LRR receptor kinase, called *LjSYMRK/MtDMI2* (Endre et al., 2002; Stracke et al., 2002). The signalling cascade then progresses via a number of genes, including those encoding potential potassium ion channels, *MtDMII*, *LjCASTOR* and *LjPOLLUX* (Ané et al., 2004; Imaizumi-Anraku et al., 2005), putative nucleoporins, *LjNUP133* and *LjNUP85* (Kanamori et al., 2006; Saito et al., 2007), a calcium–calmodulin-dependent protein kinase, *MtCCaMK* (Lévy et al., 2004; Mitra et al., 2004), a cytokinin receptor, *LjLHK1/MtCRE1* (Gonzalez-Rizzo et al., 2006; Murray et al., 2007; Oldroyd, 2007; Tirichine et al., 2007) and finally transcription factors, including *MtNSP1*, *MtNSP2*, *MtERF* and *LjNIN* (Schauser et al., 1999; Kaló et al., 2005; Smit et al., 2005; Middleton

et al., 2007). These genes are all required for nodulation; the loss of any results in reduced, or a complete lack of nodule formation.

5.4.2. Control of legume nodulation

Additional external and internal factors act as negative regulators of nodulation. Mutants unable to synthesize or perceive these factors exhibit increased nodule numbers. The best known of these factors function in the plant's Autoregulation Of Nodulation (AON) pathway (Caetano-Anollés and Gresshoff, 1991; Gresshoff, 2003; Kinkema et al., 2006; see Fig. 2B). This pathway involves long-distance root-shoot signalling initiated during nodule development by the synthesis of a root-derived signal. Grafting experiments (Delves et al., 1986; Jiang and Gresshoff, 1997) have shown this signal (named 'Q') travels to the shoot where it, or a product of its action, is perceived by a LRR receptor kinase, called *GmNARK/LjHAR1/MtSUNN* (Krusell et al., 2002; Men et al., 2002; Nishimura et al., 2002a; Searle et al., 2003; Schnabel et al., 2005). Grafting studies have also shown that the gene, *LjKLAVIER*, has a shoot-specific role in AON (Oka-Kira et al., 2005), but the identity of this gene remains unknown. Following perception in the shoot, a novel shoot-derived inhibitor (named 'SDI') is synthesized and travels back down to the roots where it acts to inhibit further nodulation events (Gresshoff and Delves, 1986). Gene chip and real time PCR analysis of leaves from inoculated or uninoculated soybean plants differing in *GmNARK* function, revealed a novel regulation of the octodecanoid pathway, suggesting jasmonic acid signaling is involved in AON (Kinkema and Gresshoff, 2008).

Root-specific AON genes have been identified in pea, *PsNOD3* (Postma et al., 1988), and *M. truncatula*, *MtRdn1* (J. Frugoli, personal communication), that are possibly involved in Q biosynthesis or translocation or SDI perception in the root.

Genes homologous to those detailed above could be identified in faba bean mutant collections (Duc and Picard, 1986; Duc, 1995), potentially leading to improved symbiosis in crop lines.

Other factors that reduce nodule numbers include ethylene and nitrate (Carroll et al., 1985a; 1985b; Ligerio et al., 1991; Lee and Larue, 1992; Ferguson and Mathesius, 2003; Ferguson et al., 2005). Mutations that disrupt the plant's ability to perceive these factors alleviate their inhibitory nature, resulting in increased nodule numbers (cf. Penmetsa and Cook, 1997). This includes genes required for ethylene sensitivity and response, such as *LjETR1* and *LjEIN2/MtEIN2* (e.g. Penmetsa et al., 2008). In addition, *nitrate-tolerant symbiosis (nts)* mutants that form many nodules when grown under inhibitory nitrate levels have been isolated in soybean and pea (Jacobsen and Feenstra, 1984; Carroll et al., 1985a; 1985b; Delves et al., 1986; Duc and Messenger, 1989), but *nts* genes not involved in AON remain to be cloned. Interestingly, the gene *LjASTRAY*, which encodes a bZIP transcription factor with a RING-finger motif, regulates light and photomorphogenic signalling and also nodulation, as loss-of-function mutant exhibit increased nodule number (Nishimura et al., 2002b). Understanding the roles of the above-mentioned regulatory genes will enable optimizing the symbiosis, resulting in tremendous agronomic impacts for faba bean.

5.4.3. Molecular genetics of nodulation and nitrogen fixation in faba bean

Faba bean forms indeterminate root nodules with the soil bacteria, *Rhizobium leguminosarum* bv. *viciae*, and in many regions where effective rhizobia populations are present, field inoculation is not practiced. Biodiversity in host-microbe populations has been exploited to improve nitrogen fixation rates of faba bean (e.g. Mytton et al., 1977; Mytton, 1984; Knaak et al., 1993). Recent molecular insights, including the

1 identification of symbiotic genes in *L. japonicus* and *M. truncatula*, will enhance these
2 classical breeding approaches. The use of faba bean mutants that fail to nodulate (Nod⁻),
3 excessively nodulate (Nod⁺⁺) even in the presence of nitrate (*nts*), or are non-functional
4 (Fix⁻, i.e., fail to fix nitrogen), including those spontaneously occurring (*sym-1*; Duc and
5 Picard, 1986; Haser et al., 1992) or chemically induced (Duc, 1995), offer further
6 potential in this area. Similarly, the identity of numerous faba bean genes encoding
7 unknown nodule proteins (nodulins) and leghemoglobins (required for nitrogen
8 fixation) (Perlick and Pühler, 1993; Frühling et al., 1997; Schröder et al., 1997; Hohnjec
9 et al., 2000; Vieweg et al., 2004) should aid in efforts to improve nitrogen fixation *via*
10 coupling transgenic techniques with classical breeding methods.

11 12 5.4.4. Application of functional genomics in faba bean

13 Advances in genomic technology and insights could aid nodulation and nitrogen
14 fixation research in faba bean. Following similar work in cereals, it was discovered that
15 legume genome maps share broad similarity (called macro-synteny; Choi et al., 2004b).
16 Likewise, molecular markers in legume genomes (usually ESTs reflecting biochemical
17 functions) are found in similar chromosomal blocks. Thus, discovery of markers linked
18 to a certain phenotype, for instance, in *M. truncatula* may provide a tool to identify the
19 same characteristic in the otherwise unexplored faba bean. It therefore becomes critical
20 that molecular linkage maps of faba bean include both ESTs and phenotypes (including
21 QTLs) relating to nodulation, nitrogen use efficiency and nitrogen fixation, and that
22 variation for these phenotypes is mapped to such conserved EST markers. The recently
23 completed, and near-completed, genome sequences of *G. max*, *M. truncatula* and *L.*
24 *japonicus* respectively, will greatly aid in this area of research

(<http://www.phytozome.net/soybean>; <http://www.medicago.org/genome/>; Sato et al., 2008).

6. Concluding Remarks

The use of model legumes to investigate important grain legume traits has already improved our knowledge on legume biology. In particular it allows important breakthroughs in our understanding of nitrogen-fixing symbiosis, and begins to bring clues to legume seed quality and resistance to biotic and abiotic stresses. The high level of synteny and conservation that exist between most legume genomes should allow an efficient transfer of all the knowledge that is being accumulated in these model legumes to improve faba bean, a grain legume for which its large genome size limits the development of post-genomic tools. Indeed, candidate genes identified by transcriptomic, proteomic or map-based cloning can be transferred to elite cultivars of faba bean after validation of its function by MAS or genetic transformation.

While the use of model legumes has already increased our knowledge on several important aspects of grain legumes, many gaps remain in our understanding of legume quality, nitrogen-fixing capacities and resistance to stresses. In parallel, biotechnological improvements allow the development of different post-genomic tools to facilitate the identification of genes and pathways, functional analysis of these genes and the search for favourable alleles in germplasm collections. Although these tools are expected to greatly help the transfer of important genes for crop improvement in a near future, they are likely to be still insufficient. For the improvement of any trait, an integration of knowledge coming from molecular biologists, plant physiologists, plant pathologists, agronomists, applied breeders and experts on social-environmental impact, involving multi-criteria decision-making programs, is required. Within the framework

of the above mentioned GLIP project some advances have been made possible. However, for real results “at the fork level”, the recently created interdisciplinary research networks, and others yet to come, need to be continued, which will require extended commitment from funding agencies at the national and international levels.

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References

Aharoni, A., Dixit, S., Jetter, R., Thoenes, E., van Arkel, G., Pereira, A., 2004. The SHINE clade of AP2 domain transcription factors activates wax biosynthesis, alters cuticle properties, and confers drought tolerance when overexpressed in Arabidopsis. Plant Cell 16, 2463-2480.

1 Ameline-Torregrosa, C., Cazaux, M., Danesh, D., Chardon, F., Cannon, S.B., Esquerré-
2 Tugayé, M.T., Dumas, B., Young, N.D., Samac, D.A., Huguet, T., Jacquet, C., 2008.
3 Genetic dissection of resistance to anthracnose and powdery mildew in *Medicago*
4 *truncatula*. Mol. Plant Microbe Interac. 21, 61-69.

5 Ané, J.M., Kiss, G.B., Riely, B.K., Penmetsa, R.V., Oldroyd, G.E.D., Ayax, C., Lévy,
6 J., Debellé, F., Baek, J.M., Kaló, P., Rosenberg, C., Roe, B.A., Long, S.R., Dénarié, J.,
7 Cook, D.R., 2004. *Medicago truncatula* DMI1 required for bacterial and fungal
8 symbioses in legumes. Science 303, 1364-1367.

9 Ané, J.M., Zhu, H., Frugoli, J., 2008. Recent Advances in *Medicago truncatula*
10 Genomics. Int. J. Plant Genomics 2008, Article ID 256597, 11 pages.
11 doi:10.1155/2008/256597.

12 Armengaud, P., Thiery, L., Buhot, N., Grenier-De March, G., Savouré, A., 2004.
13 Transcriptional regulation of proline biosynthesis in *Medicago truncatula* reveals
14 developmental and environmental specific features. Physiol. Plant. 120, 442-450.

15 Arrighi, J.F., Barre, A., Ben Amor, B., Bersoult, A., Soriano, L.C., Mirabella, R., de
16 Carvalho-Niebel, F., Journet, E.P., Ghérardi, M., Huguet, T., Geurts, R., Dénarié, J.,
17 Rougé, P., Gough, C., 2006. The *Medicago truncatula* lysin M motif-receptor-like
18 kinase gene family includes NFP and new nodule-expressed genes. Plant Physiol. 142,
19 265-279.

20 Aubert, G., Morin, J., Jacquin, F., Loridon, K., Quillet, M.C., Petit, A., Rameau, C.,
21 Lejeune-Hénaut, I., Huguet, T., Burstin, J., 2006. Functional mapping in pea, as an aid
22 to the candidate gene selection and for investigating synteny with the model legume
23 *Medicago truncatula*. Theor. Appl. Genet. 112, 1024-1041.

24 Ávila, C.M., Sillero, J.C., Rubiales, D., Moreno, M.T., Torres, A.M., 2003.
25 Identification of RAPD markers linked to the *Uvf-1* gene conferring hypersensitive

1 resistance against rust (*Uromyces viciae-fabae*) in *Vicia faba* L. Theor. Appl. Genet.
2 107, 353-358.

3 Ávila, C.M., Satovic, Z., Sillero, J.C., Rubiales, D., Moreno, M.T., Torres, A.M., 2004.
4 Isolate and organ-specific QTLs for ascochyta blight resistance in faba bean (*Vicia faba*
5 L.). Theor. Appl. Genet. 108, 1071-1078.

6 Babu, M., Gagarinova, A.G., Brandle, J.E., Wang, A., 2008. Association of the
7 transcriptional response of soybean plants with soybean mosaic virus systemic
8 infection. J. Gen. Virol. 89, 1069-1080.

9 Bailey-Serres, J., Voesenek, L., 2008. Flooding stress: Acclimations and genetic
10 diversity. Annu. Rev. Plant Biol. 59, 313-339.

11 Bañon, S., Fernández, J.A., Franco, J.A., Torrecillas, A., Alarcón, J.J., Sánchez-
12 Blanco, M.J., 2004. Effects of water stress and night temperature preconditioning on
13 water relations and morphological and anatomical changes of *Lotus creticus* plants. Sci.
14 Hortic. 101, 333-342.

15 Barbetti, M.J., 2007. Resistance in annual *Medicago spp.* to *Phoma medicaginis* and
16 *Leptosphaerulina trifolii* and its relationship to induced production of a phytoestrogen.
17 Plant Disease 91, 239-244.

18 Barbetti, M.J., Allen, J.G., 2005. Association of *Fusarium* species, with potential for
19 mycotoxicosis, on pods of annual *Medicago* in Western Australia. Austr. J. Agric. Res.
20 56, 279-284.

21 Bardel, J., Louwagie, M., Jaquinod, M., Jourdain, A., Luche, S., Rabilloud, T.,
22 Macherel, D., Garin, J., Bourguignon, J., 2002. A survey of the plant mitochondrial
23 proteome in relation to development. Proteomics 2, 880-898.

24 Barker, D.B., Bianchi, S., Blondon, F., Dattée, Y., Duc, G., Essad, S., Flament, P.,
25 Gallusci, P., Génier, P., Muel, X., Tourneur, J., Dénarié, J., Huguet, T., 1990. *Medicago*

1 *truncatula*, a model plant for studying the molecular genetics of the *Rhizobium*-legume
2 symbiosis. Plant Mol. Biol. Rep. 8, 40-49.

3 Barnett, M.J., Toman, C.J., Fisher, R.F., Long, S.R., 2004. A dual-genome Symbiosis
4 Chip for coordinate study of signal exchange and development in a prokaryote-host
5 interaction. Proc. Natl. Acad. Sci. USA 101, 16636-16641.

6 Bastianelli, D., Grosjean, F., Peyronnet, C., Duparque, M., Regnier, J.M., 1998. Feeding
7 value of pea (*Pisum sativum* L.) - 1 Chemical composition of different categories of pea.
8 Animal Science 67, 609-619.

9 Ben Amor, B., Shaw, S.L., Oldroyd, G.E.D., Maillet, F., Penmetsa, R.V., Cook, D.R.,
10 Long, S.R., Dénarié, J., Gough, C., 2003. The NFP locus of *Medicago truncatula*
11 controls an early step of Nod factor signal transduction upstream of a rapid calcium flux
12 and root hair deformation. Plant J. 34, 495-506.

13 Benedito, V.A., Torres-Jerez, I., Murray, J.D., Andriankaja, A., Allen, S., Kakar, K.,
14 Wandrey, M., Verdier, J., Zuber, H., Ott, T., Moreau, S., Niebel, A., Frickey, T.,
15 Weiller, G., He, J., Dai, X., Zhao, P.X., Tang, Y., Udvardi, M.K., 2008. A gene
16 expression atlas of the model legume *Medicago truncatula*. Plant J. 55, 504-513.

17 Bhatnagar-Mathur, P., Devi, M.J., Reddy, D.S., Lavanya, M., Vadez, V., Serraj, R.,
18 Yamaguchi-Shinozaki, K., Sharma, K.K., 2007. Stress-inducible expression of At
19 DREB1A in transgenic peanut (*Arachis hypogaea* L.) increases transpiration efficiency
20 under water-limiting conditions. Plant Cell Rep. 26, 2071-2082.

21 Borsani, O., Díaz, P., Monza, J., 1999. Proline is involved in water stress responses of
22 *Lotus corniculatus* nitrogen fixing and nitrate fed plants. J. Plant Physiol. 155, 269-273.

23 Borsani, O., Díaz, P., Agius, M.F., Valpuesta, V., Monza, J., 2001. Water stress
24 generates an oxidative stress through the induction of a specific Cu/Zn superoxide
25 dismutase in *Lotus corniculatus* leaves. Plant Sci. 161, 757-763.

1 Böttinger, P., Steinmetz, A., Schieder, O., Pickardt, T., 2001. *Agrobacterium*-mediated
2 transformation of *Vicia faba*. Mol. Breeding 8, 243-254.

3 Bourgeois, M., Jacquin, F., Savoie, V., Sommerer, N., Labas, V., Henry, C., Burstin, J.,
4 2009. Dissecting the proteome of pea mature seeds reveals the phenotypic plasticity of
5 seed protein composition. Proteomics 9, 254-271.

6 Boutin, S.R., Young, N.D., Olson, T.C., Yu, Z.H., Vallejos, C.E., Shoemaker, R.C.,
7 1995. Genome conservation among three legume genera detected with DNA markers.
8 Genome 38, 928-937.

9 Brandsaeter, L.O., Olsmo, A., Tronsmo, A.M., Fykse, H., 2002. Freezing resistance of
10 winter annual and biennial legumes at different developmental stages. Crop Sci. 42,
11 437-443.

12 Burstin, J., Marget, P., Huart, M., Moessner, A., Mangin, B., Duchene, C., Desprez, B.,
13 Munier-Jolain, N., Duc, G., 2007. Developmental genes have pleiotropic effects on
14 plant morphology and source capacity, eventually impacting on seed protein content
15 and productivity in pea. Plant Physiol. 144, 768-781.

16 Caetano-Anollés, G., Gresshoff, P.M., 1991. Plant genetic control of nodulation. Annu.
17 Rev. Microbiol. 45, 345-382.

18 Cannon, S.B., Sterck, L., Rombauts, S., Sato, S., Cheung, F., Gouzy, J., Wang, X.,
19 Mudge, J., Vasdewani, J., Schiex, T., Spannagl, M., Monaghan, E., Nicholson, C.,
20 Humphray, S.J., Schoof, H., Mayer, K.F., Rogers, J., Quetier, F., Oldroyd, G.E.D.,
21 Debellé, F., Cook, D.R., Retzel, E.F., Roe, B.A., Town, C.D., Tabata, S., Van de Peer,
22 Y., Young, N.D., 2006. Legume genome evolution viewed through the *Medicago*
23 *truncatula* and *Lotus japonicus* genomes. Proc. Natl. Acad. Sci. USA 103, 14959-
24 14964.

1 Carroll, B.J., McNeil, D.L., Gresshoff, P.M., 1985a. Isolation and properties of soybean
2 (*Glycine max* L. Merr) mutants that nodulate in the presence of high nitrate
3 concentrations. Proc. Natl. Acad. Sci. USA 82, 4162-4166.

4 Carroll, B.J., McNeil, D.L., Gresshoff, P.M., 1985b. A supernodulation and nitrate-
5 tolerant symbiotic (NTS) soybean mutant. Plant Physiol. 78, 34-40.

6 Carter, E.B., Theodorou, M.K., Morris, P., 1997. Responses of *Lotus corniculatus* to
7 environmental change. I. Effects of elevated CO₂, temperature and drought on growth
8 and plant development. New Phytol. 136, 245-253.

9 Castillejo, M.A., Maldonado, A., Rubiales, D., Jorrín, J.V., 2008. Proteomic studies of
10 legume responses to infection by *Orobanche crenata*. Proteómica 1, 134-135.

11 Castillejo, M.A., Maldonado, A.M., Dumas-Gaudot, E., Fernández-Aparicio, M., Susin,
12 R., Rubiales, D., Jorrín, J.V., 2009. Differential expression proteomics to investigate
13 responses and resistance to *Orobanche crenata* in *Medicago truncatula*. Plant Physiol.
14 submitted.

15 Chaves, M.M., Maroco, J.P., Pereira, J.S., 2003. Understanding plant responses to
16 drought: From genes to the whole plant. Funct. Plant Biol. 30, 239-264.

17 Choi, H.K., Kim, D., Uhm, T., Limpens, E., Lim, H., Mun, J.H., Kaló, P., Penmetsa,
18 R.V., Seres, A., Kulikova, O., Roe, B.A., Bisseling, T., Kiss, G.B., Cook, D.R., 2004a.
19 A sequence-based genetic map of *Medicago truncatula* and comparison of marker
20 colinearity with *M. sativa*. Genetics 166, 1463-1502.

21 Choi, H.K., Mun, J.H., Kim, D.J., Zhu, H., Baek, J.M., Mudge, J., Roe, B.A., Ellis, N.,
22 Doyle, J., Kiss, G.B., Young, N.D., Cook, D.R., 2004b. Estimating genome
23 conservation between crop and model legume species. Proc. Natl. Acad. Sci. USA 101,
24 15289-15294.

1 Colbert, T., Till, B.J., Tompa, R., Reynolds, S., Steine, M.N., Yeung, A.T., McCallum,
2 C.M., Comai, L., Henikoff, S., 2001. High-throughput screening for induced point
3 mutations. *Plant Physiol.* 126, 480-484.

4 Colditz, F., Nyamsuren, O., Niehaus, K., Eubel, H., Braun, H.P., Krajinski, F., 2004.
5 Proteomic approach: identification of *Medicago truncatula* proteins induced in roots
6 after infection with the pathogenic oomycete *Aphanomyces euteiches*. *Plant Mol. Biol.*
7 55, 109-120.

8 Colditz, F., Braun, H.P., Jacquet, C., Niehaus, K., Krajinski, F., 2005. Proteomic
9 profiling unravels insights into the molecular background underlying increased
10 *Aphanomyces euteiches*-tolerance of *Medicago truncatula*. *Plant Mol. Biol.* 59, 387-
11 406.

12 Colditz, F., Niehaus, K., Krajinski, F., 2007. Silencing of PR-10-like proteins in
13 *Medicago truncatula* results in an antagonistic induction of other PR proteins and in an
14 increased tolerance upon infection with the oomycete *Aphanomyces euteiches*. *Planta*
15 226, 57-71.

16 Colebatch, G., Desbrosses, G., Ott, T., Krusell, L., Montanari, O., Kloska, S., Kopka, J.,
17 Udvardi, M.K., 2004. Global changes in transcription orchestrate metabolic
18 differentiation during symbiotic nitrogen fixation in *Lotus japonicus*. *Plant J.* 39, 487-
19 512.

20 Constantin, G.D., Krath, B.N., MacFarlane, S.A., Nicolaisen, M., Johansen, I.E., Lund,
21 O.S., 2004. Virus-induced gene silencing as a tool for functional genomics in a legume
22 species. *Plant J.* 40, 622-631.

23 Cooper, J.L., Till, B.J., Laport, R.G., Darlow, M.C., Kleffner, J.M., Jamai, A., El-
24 Mellouki, T., Liu, S., Ritchie, R., Nielsen, N., Bilyeu, K.D., Meksem, K., Comai, L.,

1 Henikoff, S., 2008. TILLING to detect induced mutations in soybean. BMC Plant Biol.
2 8, 9.

3 Coyne, C.J., McClendon, M.T., Walling, J.G., Timmerman-Vaughan, G.M., Murray, S.,
4 Meksem, K., Lightfoot, D.A., Shultz, J.L., Keller, K.E., Martin, R.R., Inglis, D.A.,
5 Rajesh, P.N., McPhee, K.E., Weeden, N.F., Grusak, M.A., Li, C.M., Storlie, E.W.,
6 2007. Construction and characterization of two bacterial artificial chromosome libraries
7 of pea (*Pisum sativum* L.) for the isolation of economically important genes. Genome
8 50, 871-875.

9 Crane, C., Wright, E., Dixon, R.A., Wang, Z.Y., 2006. Transgenic *Medicago truncatula*
10 plants obtained from *Agrobacterium tumefaciens*-transformed roots and *Agrobacterium*
11 *rhizogenes*-transformed hairy roots. Planta 223, 1344-1354.

12 Curto, M., Ferro, N., Krajinski, F., Schlereth, A., Udvardi, M.K., Rubiales, D., 2007.
13 Real-time RT-PCR profiling of transcription factors in *Medicago truncatula* in response
14 to powdery mildew (*Erysiphe pisi*). Model Legumes Congress, Tunis, Tunisia, p. P67.

15 Dalmadi, A., Kaló, P., Jakab, J., Saskoi, A., Petrovics, T., Deák, G., Kiss, G.B., 2008.
16 Dwarf plants of diploid *Medicago sativa* carry a mutation in the gibberellin 3-beta-
17 hydroxylase gene. Plant Cell Rep. 27, 1271-1279.

18 Dalmais, M., Schmidt, J., Le Signor, C., Moussy, F., Burstin, J., Savoie, V., Aubert, G.,
19 Brunaud, V., de Oliveira, Y., Guichard, C., Thompson, R., Bendahmane, A., 2008.
20 UTILLdb, a *Pisum sativum* in silico forward and reverse genetics tool. Genome Biol. 9,
21 R43.

22 de Lorenzo, L., Merchan, F., Blanchet, S., Megías, M., Frugier, F., Crespi, M., Sousa,
23 C., 2007. Differential expression of the TFIIIA regulatory pathway in response to salt
24 stress between *Medicago truncatula* genotypes. Plant Physiol. 145, 1521-1532.

1 De Smet, I., Jurgens, G., 2007. Patterning the axis in plants-auxin in control. Curr.
2 Opin. Genet. Dev. 17, 337-343.

3 Delves, A.C., Mathews, A., Day, D.A., Carter, A.S., Carroll, B.J., Gresshoff, P.M.,
4 1986. Regulation of the soybean-*Rhizobium* nodule symbiosis by shoot and root factors.
5 Plant Physiol. 82, 588-590.

6 Devos, K.M., Beales, J., Nagamura, Y., Sasaki, T., 1999. Arabidopsis-rice: Will
7 colinearity allow gene prediction across the eudicot-monocot divide? Genome Res. 9,
8 825-829.

9 Díaz, P., Monza, J., Márquez, A., 2005. Drought and saline stress. In: Márquez, A.J.,
10 Stougaard, J., Udvardi, M.K., Parniske, M., Spaink, H.P., Saalbach, G., Webb, K.J.,
11 Chiurazzi, M. (Eds.), *Lotus Japonicus* Handbook, Springer, Dordrecht, Germany, pp39-
12 50.

13 Dié, J.V., Dita, M.A., Krajinski, F., González-Verdejo, C.I., Rubiales, D., Moreno,
14 M.T., Román, B., 2007. Identification by suppression subtractive hybridization and
15 expression analysis of *Medicago truncatula* putative defence genes in response to
16 *Orobanche crenata* parasitization. Physiol. Mol. Plant Pathol. 70, 49-59.

17 Dita, M.A., Dié, J.V., Román, B., Krajinski, F., Küster, H., Moreno, M.T., Cubero, J.I.,
18 Rubiales, D., 2009. Gene expression profiling of *Medicago truncatula* roots in response
19 to the parasitic plant *Orobanche crenata*. Planta submitted.

20 Djemel, N., Guedon, D., Lechevalier, A., Salon, C., Miquel, M., Prosperi, J.M., Rochat,
21 C., Boutin, J.P., 2005. Development and composition of the seeds of nine genotypes of
22 the *Medicago truncatula* species complex. Plant Physiol. Biochem. 43, 557-566.

23 Duc, G., 1995. Mutagenesis of faba bean (*Vicia faba* L.) and the identificaion of 5
24 different genes controlling no nodulation, ineffective nodulation or supernodulation.
25 Euphytica 83, 147-152.

1 Duc, G., 2004. Seed composition of *Medicago truncatula* (line J5), compared to other
2 seed legumes. 5th European Conference on grain legumes/ 2nd ICLGG Conference,
3 Dijon, France, p 404.

4 Duc, G., Picard, J., 1986. Note on the presence of the *sym1* gene in *Vicia faba*
5 hampering its symbiosis with *Rhizobium leguminosarum*. Euphytica 35, 61-64.

6 Duc, G., Messenger, A., 1989. Mutagenesis of pea (*Pisum sativum* L.) and the isolation
7 of mutants for nodulation and nitrogen-fixation. Plant Sci. 60, 207-213.

8 Duc, G., Marget, P., Esnault, R., Le Guen, J., Bastianelli, D., 1999. Genetic variability
9 for feeding value of faba bean seeds (*Vicia faba*): Comparative chemical composition of
10 isogenics involving zero-tannin and zero-vicine genes. J. Agric. Sci. 133, 185-196.

11 El Yahyaoui, F., Küster, H., Ben Amor, B., Hohnjec, N., Pühler, A., Becker, A., Gouzy,
12 J., Vernié, T., Gough, C., Niebel, A., Godiard, L., Gamas, P., 2004. Expression profiling
13 in *Medicago truncatula* identifies more than 750 genes differentially expressed during
14 nodulation, including many potential regulators of the symbiotic program. Plant
15 Physiol. 136, 3159-3176.

16 Ellis, T.H.N., Poyser, S.J., 2002. An integrated and comparative view of pea genetic and
17 cytogenetic maps. New Phytol. 153, 17-25.

18 Ellwood, S.R., Kamphuis, L.G., Oliver, R.P., 2006. Identification of sources of
19 resistance to *Phoma medicaginis* isolates in *Medicago truncatula* SARDI core
20 collection accessions, and multigene differentiation of isolates. Phytopathology 96,
21 1330-1336.

22 Endre, G., Kereszt, A., Kevei, Z., Mihacea, S., Kaló, P., Kiss, G.B., 2002. A receptor
23 kinase gene regulating symbiotic nodule development. Nature 417, 962-966.

24 Ferguson, B.J., Mathesius, U., 2003. Signaling interactions during nodule development.
25 J. Plant Growth Regul. 22, 47-72.

1 Ferguson, B.J., Wiebe, E.M.K., Emery, R.J.N., Guinel, F.C., 2005. Cytokinin
2 accumulation and an altered ethylene response mediate the pleiotropic phenotype of the
3 pea nodulation mutant R50 (*sym16*). Can. J. Bot. 83, 989-1000.

4 Fernández-Aparicio, M., Pérez-De-Luque, A., Prats, E., Rubiales, D., 2008. Variability
5 of interactions between barrel medic (*Medicago truncatula*) genotypes and *Orobanche*
6 species. Ann. Appl. Biol. 153, 117-126.

7 Fernández-Aparicio, M., Flores, F., Rubiales, D., 2009. Recognition of root exudates by
8 seeds of broomrape (*Orobanche* and *Phelipanche*) species. Ann. Bot. 103, 423-431.

9 Fondevilla, S., Krajinski, F., Küster, H., Torres, A.M., Moreno, M.T., Rubiales, D.,
10 2008. Identificación de genes expresados diferencialmente en la resistencia a
11 *Mycosphaerella pinodes* en guisante. Conference SEG, Cordoba, Spain.

12 Foster-Hartnett, D., Danesh, D., Penuela, S., Sharopova, N., Endre, G., Vandenbosch,
13 K.A., Young, N.D., Samac, D.A., 2007. Molecular and cytological responses of
14 *Medicago truncatula* to *Erysiphe pisi*. Mol. Plant Pathol. 8, 307-319.

15 Frühling, M., Roussel, H., Gianinazzi-Pearson, V., Pühler, A., Perlick, A.M., 1997. The
16 *Vicia faba* leghemoglobin gene *VfLb29* is induced in root nodules and in roots colonized
17 by the arbuscular mycorrhizal fungus *Glomus fasciculatum*. Mol. Plant Microbe Interac.
18 10, 124-131.

19 Furutani, N., Yamagishi, N., Hidaka, S., Shizukawa, Y., Kanematsu, S., Kosaka, Y.,
20 2007. Soybean mosaic virus resistance in transgenic soybean caused by post-
21 transcriptional gene silencing. Breeding Sci. 57, 123-128.

22 Gálvez, L., González, E.M., Arrese-Igor, C., 2005. Evidence for carbon flux shortage
23 and strong carbon/nitrogen interactions in pea nodules at early stages of water stress. J.
24 Exp. Bot. 56, 2551-2561.

1 Gallardo, K., Le Signor, C., Vandekerckhove, J., Thompson, R.D., Burstin, J., 2003.
2 Proteomics of *Medicago truncatula* seed development establishes the time frame of
3 diverse metabolic processes related to reserve accumulation. Plant Physiol. 133, 664-
4 682.

5 Gallardo, K., Kurt, C., Thompson, R., Ochatt, S., 2006. In vitro culture of immature *M.*
6 *truncatula* grains under conditions permitting embryo development comparable to that
7 observed *in vivo*. Plant Sci. 170, 1052-1058.

8 Gallardo, K., Firnhaber, C., Zuber, H., Hericher, D., Belghazi, M., Henry, C., Küster,
9 H., Thompson, R., 2007. A combined proteome and transcriptome analysis of
10 developing *Medicago truncatula* seeds: Evidence for metabolic specialization of
11 maternal and filial tissues. Mol. Cell Proteomics 6, 2165-2179.

12 Gonzales, M.D., Archuleta, E., Farmer, A., Gajendran, K., Grant, D., Shoemaker, R.C.,
13 Beavis, W.D., Waugh, M.E., 2005. The Legume Information System (LIS): An
14 integrated information resource for comparative legume biology. Nucleic Acids Res. 33,
15 660-665.

16 Gonzalez-Rizzo, S., Crespi, M., Frugier, F., 2006. The *Medicago truncatula* CRE1
17 cytokinin receptor regulates lateral root development and early symbiotic interaction
18 with *Sinorhizobium meliloti*. Plant Cell 18, 2680-2693.

19 González, E.M., Gordon, A.J., James, C.L., Arrese-Igor, C., 1995. The role of sucrose
20 synthase in the response of soybean nodules to drought. J. Exp. Bot. 46, 1515-1523.

21 González, E.M., Aparicio-Tejo, P.M., Gordon, A.J., Minchin, F.R., Royuela, M.,
22 Arrese-Igor, C., 1998. Water-deficit effects on carbon and nitrogen metabolism of pea
23 nodules. J. Exp. Bot. 49, 1705-1714.

1 Greene, S., Hughes, S.J., Nair, R., Huguet, T., Aouani, M.E., Prosperi, J.M., Delalande,
2 M., 2006. Wild accessions/populations. In: Mathesius, U., Journet, E.P., Sumner, L.W.
3 (Eds.), The *Medicago truncatula* handbook. <http://www.noble.org/MedicagoHandbook>)
4 Gresshoff, P.M., 2003. Post-genomic insights into plant nodulation symbioses. *Genome*
5 *Biol.* 4, 201.
6 Gresshoff, P.M., Delves, A.C., 1986. Plant genetic approaches to symbiotic nodulation
7 and nitrogen fixation in legumes. *Plant Gene Res.* 3, 159-206.
8 Gruber, V., Blanchet, S., Diet, A., Zahaf, O., Boualem, A., Kakar, K., Alunni, B.,
9 Udvardi, M.K., Frugier, F., Crespi, M., 2009. Identification of transcription factors
10 involved in root apex responses to salt stress in *Medicago truncatula*. *Mol. Genet.*
11 *Genomics* 1, 55-66.
12 Gutiérrez, N., Ávila, C.M., Duc, G., Marget, P., Suso, M.J., Moreno, M.T., Torres,
13 A.M., 2006. CAPs markers to assist selection for low vicine and convicine contents in
14 faba bean (*Vicia faba* L.). *Theor. Appl. Genet.* 114, 59-66.
15 Gutiérrez, N., Ávila, C.M., Rodríguez-Suárez, C., Moreno, M.T., Torres, A.M., 2007.
16 Development of SCAR markers linked to a gene controlling absence of tannins in faba
17 bean. *Mol. Breeding* 19, 305-314.
18 Gutiérrez, N., Ávila, C.M., Moreno, M.T., Torres, A.M., 2008a. Development of SCAR
19 markers linked to zt-2, one of the genes controlling absence of tannins in faba bean.
20 *Austr. J. Agric. Res.* 59, 62-68.
21 Gutiérrez, N., Palomino, C., Cruz-Izquierdo, S., Ávila, C.M., Torres, A.M., 2008b.
22 Empleo de la genómica comparativa em la mejora de las habas (*Vicia faba*). *Actas*
23 *Horticultura* 51, pp. 269-270.
24 Handberg, K., Stougaard, J., 1992. *Lotus japonicus*, an autogamous, diploid legume
25 species for classical and molecular genetics. *Plant J.* 2, 487-496.

1 Hasegawa, P.M., Bressan, R.A., Zhu, J.K., Bohnert, H.J., 2000. Plant cellular and
2 molecular responses to high salinity. *Annu. Rev. Plant Phys.* 51, 463-499.

3 Haser, A., Robinson, D.L., Duc, G., Vance, C.P., 1992. A mutation in *Vicia faba* results
4 in ineffective nodules with impaired bacteroid differentiation and reduced synthesis of
5 late nodulins. *J. Exp. Bot.* 43, 1397-1407.

6 Hayashi, S., Gresshoff, P.M., Kinkema, M., 2008. Molecular analysis of lipxygenases
7 associated with nodule development in soybean. *Mol. Plant Microbe Interac.* 21, 843-
8 853.

9 Heckmann, A.B., Lombardo, F., Miwa, H., Perry, J.A., Bunnewell, S., Parniske, M.,
10 Wang, T.L., Downie, J.A., 2006. *Lotus japonicus* nodulation requires two GRAS
11 domain regulators, one of which is functionally conserved in a non-legume. *Plant*
12 *Physiol.* 142, 1739-1750.

13 Heino, P., Palva, E.T., 2003. Signal transduction in plant cold acclimation. In: H. Hirt,
14 K. Shinozaki (Eds.) *Plant responses to abiotic stress. Topics in Current Genetics* volume
15 4. Springer-Verlag, Berlin, pp. 151-186.

16 Hekneby, M., Antolin, M.C., Sanchez-Diaz, M., 2006. Frost resistance and biochemical
17 changes during cold acclimation in different annual legumes. *Environ. Exp. Bot.* 55,
18 305-314.

19 Hoffmann, D., Jiang, Q., Men, A., Kinkema, M., Gresshoff, P.M., 2007. Nodulation
20 deficiency caused by fast neutron mutagenesis of the model legume *Lotus japonicus*. *J.*
21 *Plant Physiol.* 164, 460-469.

22 Hohnjec, N., Küster, H., Albus, U., Frosch, S.C., Becker, J.D., Pühler, A., Perlick,
23 A.M., Fröhling, M., 2000. The broad bean nodulin VfENOD18 is a member of a novel
24 family of plant proteins with homologies to the bacterial MJ0577 superfamily. *Mol.*
25 *Gen. Genet.* 264, 241-250.

1 Hohnjec, N., Vieweg, M.F., Pühler, A., Becker, A., Küster, H., 2005. Overlaps in the
2 transcriptional profiles of *Medicago truncatula* roots inoculated with two different
3 *Glomus* fungi provide insights into the genetic program activated during arbuscular
4 mycorrhiza. *Plant Physiol.* 137, 1283-1301.

5 Horst, I., Welham, T., Kelly, S., Kaneko, T., Sato, S., Tabata, S., Parniske, M., Wang,
6 T.L., 2007. TILLING mutants of *Lotus japonicus* reveal that nitrogen assimilation and
7 fixation can occur in the absence of nodule-enhanced sucrose synthase. *Plant Physiol.*
8 144, 806-820.

9 Hougaard, B.K., Madsen, L.H., Sandal, N., de Carvalho Moretzsohn, M., Fredslund, J.,
10 Schauser, L., Nielsen, A.M., Rohde, T., Sato, S., Tabata, S., Bertoli, D.J., Stougaard, J.,
11 2008. Legume anchor markers link syntenic regions between *Phaseolus vulgaris*, *Lotus*
12 *japonicus*, *Medicago truncatula* and *Arachis*. *Genetics* 179, 2299-2312.

13 Humphry, M.E., Konduri, V., Lambrides, C.J., Magner, T., McIntyre, C.L., Aitken,
14 E.A.B., Liu, C.J., 2002. Development of a mungbean (*Vigna radiata*) RFLP linkage
15 map and its comparison with lablab (*Lablab purpureus*) reveals a high level of
16 colinearity between the two genomes. *Theor. Appl. Genet.* 105, 160-166.

17 Imaizumi-Anraku, H., Takeda, N., Charpentier, M., Perry, J., Miwa, H., Umehara, Y.,
18 Kouchi, H., Murakami, Y., Mulder, L., Vickers, K., Pike, J., Downie, J.A., Wang, T.,
19 Sato, S., Asamizu, E., Tabata, S., Yoshikawa, M., Murooka, Y., Wu, G.J., Kawaguchi,
20 M., Kawasaki, S., Parniske, M., Hayashi, M., 2005. Plastid proteins crucial for
21 symbiotic fungal and bacterial entry into plant roots. *Nature* 433, 527-531.

22 Jacobsen, E., Feenstra, W.J., 1984. A new pea mutant with efficient nodulation in the
23 presence of nitrate. *Plant Sci. Lett.* 33, 337-344.

24 Jiang, Q.Y., Gresshoff, P.M., 1997. Classical and molecular genetics of the model
25 legume *Lotus japonicus*. *Mol. Plant Microbe Interac.* 10, 59-68.

1 Jones, H., 2004. What is water use efficiency? In: Bacon, M.A. (Ed.), Water use
2 efficiency in plant biology. Blackwell Publishing, oxford, pp. 27-41.

3 Jones, J.D.G., Dangl, J.L., 2006. The plant immune system. *Nature* 444, 323-329.

4 Julier, B., Huguet, T., Chardon, F., Ayadi, R., Pierre, J.B., Prosperi, J.M., Barre, P.,
5 Huyghe, C., 2007. Identification of quantitative trait loci influencing aerial
6 morphogenesis in the model legume *Medicago truncatula*. *Theor. Appl. Genet.* 114,
7 1391-1406.

8 Kachroo, A., Fu, D.Q., Havens, W., Navarre, D., Kachroo, P., Ghabrial, S.A., 2008. An
9 oleic acid-mediated pathway induces constitutive defense signaling and enhanced
10 resistance to multiple pathogens in soybean. *Mol. Plant Microbe Interac.* 21, 564-575.

11 Kaga, A., Ishii, T., Tsukimoto, K., Tokoro, E., Kamijima, O., 2000. Comparative
12 molecular mapping in *Ceratotropis* species using an interspecific cross between azuki
13 bean (*Vigna angularis*) and rice bean (*V. umbellata*). *Theor. Appl. Genet.* 100, 207-213.

14 Kaimoyo, E., VanEtten, H.D., 2008. Inactivation of pea genes by RNAi supports the
15 involvement of two similar O-methyltransferases in the biosynthesis of (+)-pisatin and
16 of chiral intermediates with a configuration opposite that found in (+)-pisatin.
17 *Phytochemistry* 69, 76-87.

18 Kakar, K., Wandrey, M., Czechowski, T., Gaertner, T., Scheible, W.R., Stitt, M.,
19 Torres-Jerez, I., Xiao, Y., Redman, J.C., Wu, H.C., Cheung, F., Town, C.D., Udvardi,
20 M.K., 2008. A community resource for high-throughput quantitative RT-PCR analysis
21 of transcription factor gene expression in *Medicago truncatula*. *Plant Methods* 4, 18.

22 Kaló, P., Endre, G., Zimányi, L., Csanádi, G., Kiss, G.B., 2000. Construction of an
23 improved linkage map of diploid alfalfa (*Medicago sativa*). *Theor. Appl. Genet.* 100,
24 641-657.

1 Kaló, P., Seres, A., Taylor, S.A., Jakab, J., Kevei, Z., Kereszt, A., Endre, G., Ellis, T.H.,
 2 Kiss, G.B., 2004. Comparative mapping between *Medicago sativa* and *Pisum sativum*.
 3 Mol. Genet. Genomics 272, 235-246.
 4 Kaló, P., Gleason, C., Edwards, A., Marsh, J., Mitra, R.M., Hirsch, S., Jakab, J., Sims,
 5 S., Long, S.R., Rogers, J., Kiss, G.B., Downie, J.A., Oldroyd, G.E.D., 2005. Nodulation
 6 signaling in legumes requires NSP2, a member of the GRAS family of transcriptional
 7 regulators. Science 308, 1786-1789.
 8 Kamphuis, L.G., Lichtenzveig, J., Oliver, R.P., Ellwood, S.R., 2008. Two alternative
 9 recessive quantitative trait loci influence resistance to spring black stem and leaf spot in
 10 *Medicago truncatula*. BMC Plant Biol. 8, 30.
 11 Kanamori, N., Madsen, L.H., Radutoiu, S., Frantescu, M., Quistgaard, E.M., Miwa, H.,
 12 Downie, J.A., James, E.K., Felle, H.H., Haaning, L.L., Jensen, T.H., Sato, S.,
 13 Nakamura, Y., Tabata, S., Sandal, N., Stougaard, J., 2006. A nucleoporin is required for
 14 induction of Ca²⁺ spiking in legume nodule development and essential for rhizobial and
 15 fungal symbiosis. Proc. Natl. Acad. Sci. USA 103, 359-364.
 16 Kawaguchi, M., Imaizumi-Anraku, H., Koiwa, H., Niwa, S., Ikuta, A., Syono, K., Akao,
 17 S., 2002. Root, root hair, and symbiotic mutants of the model legume *Lotus japonicus*.
 18 Mol. Plant Microbe Interac. 15, 17-26.
 19 Kereszt, A., Li, D., Indrasumunar, A., Nguyen, C.D., Nontachaiyapoom, S., Kinkema,
 20 M., Gresshoff, P.M., 2007. *Agrobacterium rhizogenes*-mediated transformation of
 21 soybean to study root biology. Nat. Protoc. 2, 948-952.
 22 Kevei, Z., Seres, A., Kereszt, A., Kaló, P., Kiss, P., Tóth, G., Endre, G., Kiss, G.B.,
 23 2005. Significant microsynteny with new evolutionary highlights is detected between
 24 Arabidopsis and legume model plants despite the lack of macrosynteny. Mol. Genet.
 25 Genomics 274, 644-657.

1 Kinkema, M., Gresshoff, P.M., 2008. Investigation of downstream signals of the
2 soybean autoregulation of nodulation receptor kinase GmNARK. *Mol. Plant Microbe*
3 *Interac.* 21, 1337-1348.

4 Kinkema, M., Scott, P.T., Gresshoff, P.M., 2006. Legume nodulation: successful
5 symbiosis through short- and long-distance signalling. *Funct. Plant Biol.* 33, 707-721.

6 Knaak, C., Roskothen, P., Röbbelen, G., 1993. Symbiotic efficiency of *Vicia faba*
7 genotypes after field inoculation with different strains of *Rhizobium leguminosarum*
8 preselected in greenhouse tests. *J. Plant Physiol.* 141, 49-53.

9 Kouchi, H., Shimomura, K., Hata, S., Hirota, A., Wu, G.J., Kumagai, H., Tajima, S.,
10 Suganuma, N., Suzuki, A., Aoki, T., Hayashi, M., Yokoyama, T., Ohyama, T.,
11 Asamizu, E., Kuwata, C., Shibata, D., Tabata, S., 2004. Large-scale analysis of gene
12 expression profiles during early stages of root nodule formation in a model legume,
13 *Lotus japonicus*. *DNA Res.* 11, 263-274.

14 Krusell, L., Madsen, L.H., Sato, S., Aubert, G., Genua, A., Szczygłowski, K., Duc, G.,
15 Kaneko, T., Tabata, S., de Bruijn, F., Pajuelo, E., Sandal, N., Stougaard, J., 2002. Shoot
16 control of root development and nodulation is mediated by a receptor-like kinase.
17 *Nature* 420, 422-426.

18 Kubo, M., Ueda, H., Park, P., Kawaguchi, M., Sugimoto, Y., 2009. Reactions of *Lotus*
19 *japonicus* ecotypes and mutants to root parasitic plants. *J. Plant Physiol.* 166, 353-362

20 Kulikova, O., Gualtieri, G., Geurts, R., Kim, D.J., Cook, D.R., Huguet, T., de Jong,
21 J.H., Fransz, P.F., Bisseling, T., 2001. Integration of the FISH pachytene and genetic
22 maps of *Medicago truncatula*. *Plant J.* 27, 49-58.

23 Küster, H., Hohnjec, N., Krajinski, F., El, Y.F., Manthey, K., Gouzy, J., Dondrup, M.,
24 Meyer, F., Kalinowski, J., Brechenmacher, L., van Tuinen, D., Gianinazzi-Pearson, V.,
25 Pühler, A., Gamas, P., Becker, A., 2004. Construction and validation of cDNA-based

1 Mt6k-RIT macro- and microarrays to explore root endosymbioses in the model legume
2 *Medicago truncatula*. J. Biotechnol. 108, 95-113.

3 Larrainzar, E., Wienkoop, S., Weckwerth, W., Ladrera, R., Arrese-Igor, C., González,
4 E.M., 2007. *Medicago truncatula* root nodule proteome analysis reveals differential
5 plant and bacteroid responses to drought stress. Plant Physiol. 144, 1495-1507.

6 Le, B.H., Wagmaister, J.A., Kawashima, T., Bui, A.Q., Harada, J.J., Goldberg, R.B.,
7 2007. Using genomics to study legume seed development. Plant Physiol. 144, 562-574.

8 Le Signor, C., Gallardo, K., Prosperi, J.M., Salon, C., Quillien, L., Thompson, R., Duc,
9 G., 2005. Genetic diversity for seed protein composition in *Medicago truncatula*. Plant
10 Genet. Resour. 3, 59-71.

11 Lee, H.S., Kim, H.J., An, C.S., 2004. Cloning and expression analysis of 2-on-2
12 hemoglobin from soybean. J. Plant Biol. 47, 92-98.

13 Lee, J.M., Grant, D., Vallejos, C.E., Shoemaker, R.C., 2001. Genome organization in
14 dicots. II. Arabidopsis as a “bridging species” to resolve genome evolution events
15 among legumes. Theor. Appl. Genet. 103, 765-773.

16 Lee, K.H., Larue, T.A., 1992. Ethylene as a possible mediator of light-induced and
17 nitrate-induced inhibition of nodulation of *Pisum sativum* L. cv sparkle. Plant Physiol.
18 100, 1334-1338.

19 Lejeune-Hénaut, I., Hanocq, E., Béthencourt, L., Fontaine, V., Delbreil, B., Morin, J.,
20 Petit, A., Devaux, R., Boilleau, M., Stempniak, J.J., Thomas, M., Lainé, A.L., Foucher,
21 F., Baranger, A., Burstin, J., Rameau, C., Giauffret, C., 2008. The flowering locus *Hr*
22 colocalizes with a major QTL affecting winter frost tolerance in *Pisum sativum* L.
23 Theor. Appl. Genet. 116, 1105-1116.

24 Lemontey, C., Mousset-Declas, C., Munier-Jolain, N., Boutin, J.P., 2000. Maternal
25 genotype influences pea seed size by controlling both mitotic activity during early

1 embryogenesis and final endoreduplication level/cotyledon cell size in mature seed. J.
2 Exp. Bot. 51, 167-175.

3 Lévy, J., Bres, C., Geurts, R., Chalhoub, B., Kulikova, O., Duc, G., Journet, E.P., Ané,
4 J.M., Lauber, E., Bisseling, T., Dénarié, J., Rosenberg, C., Debellé, F., 2004. A putative
5 Ca^{2+} and calmodulin-dependent protein kinase required for bacterial and fungal
6 symbioses. Science 303, 1361-1364.

7 Ligeró, F., Caba, J.M., Lluch, C., Olivares, J., 1991. Nitrate inhibition of nodulation can
8 be overcome by the ethylene inhibitor aminoethoxyvinylglycine. Plant Physiol. 97,
9 1221-1225.

10 Limami, A.M., Ricoult, C., Planchet, E., González, E.M., Ladrera, R., Larrainzar, E.,
11 Arrese-Igor, C., Merchan, F., Crespi, M., Frugier, F., 2006. Response of *Medicago*
12 *truncatula* to abiotic stress. In: Mathesius, U., Journet, E.P., Sumner, L.W. (Eds.), The
13 *Medicago truncatula* handbook. (<http://www.noble.org/MedicagoHandbook>)

14 Limpens, E., Franken, C., Smit, P., Willemse, J., Bisseling, T., Geurts, R., 2003. LysM
15 domain receptor kinases regulating rhizobial Nod factor-induced infection. Science 302,
16 630-633.

17 Limpens, E., Ramos, J., Franken, C., Raz, V., Compaan, B., Franssen, H., Bisseling, T.,
18 Geurts, R., 2004. RNA interference in *Agrobacterium rhizogenes*-transformed roots of
19 *Arabidopsis* and *Medicago truncatula*. J. Exp. Bot. 55, 983-992.

20 Lombardi, P., Ercolano, E., El Alaoui, H., Chiurazzi, M., 2003. A new transformation-
21 regeneration procedure in the model legume *Lotus japonicus*: root explants as a source
22 of large numbers of cells susceptible to *Agrobacterium*-mediated transformation. Plant
23 Cell Rep. 21, 771-777.

1 López, M., Herrera-Cervera, J.A., Iribarne, C., Tejera, N.A., Lluch, C., 2008. Growth
 2 and nitrogen fixation in *Lotus japonicus* and *Medicago truncatula* under NaCl stress:
 3 Nodule carbon metabolism. J. Plant Physiol. 165, 641-650.
 4 Loridon, K., McPhee, K., Morin, J., Dubreuil, P., Pilet-Nayel, M.L., Aubert, G.,
 5 Rameau, C., Baranger, A., Coyne, C., Lejeune-Hénaut, I., Burstin, J., 2005.
 6 Microsatellite marker polymorphism and mapping in pea (*Pisum sativum* L.). Theor.
 7 Appl. Genet. 111, 1022-1031.
 8 Lozano-Baena, M.D., Prats, E., Moreno, M.T., Rubiales, D., Pérez-de-Luque, A., 2007.
 9 *Medicago truncatula* as a model for nonhost resistance in legume-parasitic plant
 10 interactions. Plant Physiol. 145, 437-449.
 11 Ma, W., Smigel, A., Tsai, Y.C., Braam, J., Berkowitz, G.A., 2008. Innate immunity
 12 signaling: Cytosolic Ca²⁺ elevation is linked to downstream nitric oxide generation
 13 through the action of calmodulin or a calmodulin-like protein. Plant Physiol. 148, 818-
 14 828.
 15 Madrid, E., Gil, J., Krajinski, F., Schlereth, A., Rubiales, D., 2008. Perfil de expresión
 16 de factores de transcripción en la interacción interacción *Medicago truncatula*-
 17 *Uromyces striatus*. Conference SEG, Cordoba, Spain.
 18 Madsen, E.B., Madsen, L.H., Radutoiu, S., Olbryt, M., Rakwalska, M., Szczygłowski,
 19 K., Sato, S., Kaneko, T., Tabata, S., Sandal, N., Stougaard, J., 2003. A receptor kinase
 20 gene of the LysM type is involved in legume perception of rhizobial signals. Nature
 21 425, 637-640.
 22 Maeda, D., Ashida, K., Iguchi, K., Chechetka, S.A., Hijikata, A., Okusako, Y., Deguchi,
 23 Y., Izui, K., Hata, S., 2006. Knockdown of an arbuscular mycorrhiza-inducible
 24 phosphate transporter gene of *Lotus japonicus* suppresses mutualistic symbiosis. Plant
 25 Cell Physiol 47, 807-817.

1 Marino, D., Hohnjec, N., Küster, H., Moran, J.F., González, E.M., Arrese-Igor, C.,
2 2008. Evidence for transcriptional and post-translational regulation of sucrose synthase
3 in pea nodules by the cellular redox state. *Mol. Plant Microbe Interac.* 21, 622-630.

4 Maroof, M.A.S., Jeong, S.C., Gunduz, I., Tucker, D.M., Buss, G.R., Tolin, S.A., 2008a.
5 Pyramiding of soybean mosaic virus resistance genes by marker-assisted selection. *Crop*
6 *Sci.* 48, 517-526.

7 Maroof, M.A.S., Tucker, D.M., Tolin, S.A., 2008b. Genomics of viral-soybean
8 interactions. *Gen. Genomics Soybean*, 293-319.

9 Mathews, A., Carroll, B.J., Gresshoff, P.M., 1989. Development of *Bradyrhizobium*
10 infections in supernodulating and non-nodulating mutants of soybean (*Glycine max* L.
11 Merrill). *Protoplasma* 150, 40-47.

12 McKersie, B.D., Chen, Y.R., Debeus, M., Bowley, S.R., Bowler, C., Inzé, D., Dhalluin,
13 K., Botterman, J., 1993. Superoxide dismutase enhances tolerance of freezing stress in
14 transgenic alfalfa (*Medicago sativa* L.). *Plant Physiol.* 103, 1155-1163.

15 McKersie, B.D., Bowley, S.R., Harjanto, E., Leprince, O., 1996. Water-deficit tolerance
16 and field performance of transgenic alfalfa overexpressing superoxide dismutase. *Plant*
17 *Physiol.* 111, 1177-1181.

18 McKersie, B.D., Murnaghan, J., Jones, K.S., Bowley, S.R., 2000. Iron-superoxide
19 dismutase expression in transgenic alfalfa increases winter survival without a detectable
20 increase in photosynthetic oxidative stress tolerance. *Plant Physiol.* 122, 1427-1437.

21 Meixner, C., Vegvari, G., Ludwig-Müller, J., Gagnon, H., Steinkellner, S., Staehelin,
22 C., Gresshoff, P., Vierheilig, H., 2007. Two defined alleles of the LRR receptor kinase
23 GmNARK in supernodulating soybean govern differing autoregulation of
24 mycorrhization. *Physiol. Plant.* 130, 261-270.

1 Men, A.E., Laniya, T.S., Searle, I.R., Iturbe-Ormaetxe, I., Hussain, A.K.M., Gresshoff,
2 I., Jiang, Q., Carroll, B.J., Gresshoff, P.M., 2002. Fast neutron mutagenesis of soybean
3 (*Glycine soja* [L.]) produces a supernodulating mutant containing a large deletion.
4 Genome Lett. 3, 147-155.

5 Menancio-hautea, D., Fatokun, C.A., Kumar, L., Danesh, D., Young, N.D., 1993.
6 Comparative genome analysis of mungbean (*Vigna radiata* L. Wilczek) and cowpea (*V.*
7 *unguiculata* L. Walpers) using RFLP mapping data. Theor. Appl. Genet. 86, 797-810.

8 Merchan, F., Breda, C., Hormaeche, J.P., Sousa, C., Kondorosi, A., Aguilar, O.M.,
9 Megias, M., Crespi, M., 2003. A Kruppel-like transcription factor gene is involved in
10 salt stress responses in *Medicago spp.* Plant Soil 257, 1-9.

11 Merchan, F., de Lorenzo, L., Rizzo, S.G., Niebel, A., Manyani, H., Frugier, F., Sousa,
12 C., Crespi, M., 2007. Identification of regulatory pathways involved in the reacquisition
13 of root growth after salt stress in *Medicago truncatula*. Plant J. 51, 1-17.

14 Middleton, P.H., Jakab, J., Penmetsa, R.V., Starker, C.G., Doll, J., Kaló, P., Prabhu, R.,
15 Marsh, J.F., Mitra, R.M., Kereszt, A., Dudas, B., VandenBosch, K., Long, S.R., Cook,
16 D.R., Kiss, G.B., Oldroyd, G.E.D., 2007. An ERF transcription factor in *Medicago*
17 *truncatula* that is essential for Nod factor signal transduction. Plant Cell 19, 1221-1234.

18 Mitra, R.M., Gleason, C.A., Edwards, A., Hadfield, J., Downie, J.A., Oldroyd, G.E.D.,
19 Long, S.R., 2004. A Ca²⁺/calmodulin-dependent protein kinase required for symbiotic
20 nodule development: Gene identification by transcript-based cloning. Proc. Natl. Acad.
21 Sci. USA 101, 4701-4705.

22 Moussart, A., Onfroy, C., Lesne, A., Esquibet, M., Grenier, E., Tivoli, B., 2007. Host
23 status and reaction of *Medicago truncatula* accessions to infection by three major
24 pathogens of pea (*Pisum sativum*) and alfalfa (*Medicago sativa*). Eur. J. plant Pathol.
25 117, 57-69.

1 Mudge, J., Cannon, S.B., Kaló, P., Oldroyd, G.E.D., Roe, B.A., Town, C.D., Young,
2 N.D., 2005. Highly syntenic regions in the genomes of soybean, *Medicago truncatula*,
3 and *Arabidopsis thaliana*. BMC Plant Biol. 5, 15.

4 Murray, J.D., Karas, B.J., Sato, S., Tabata, S., Amyot, L., Szczygłowski, K., 2007. A
5 cytokinin perception mutant colonized by *Rhizobium* in the absence of nodule
6 organogenesis. Science 315, 101-104.

7 Mytton, L.R., 1984. Developing a breeding strategy to exploit quantitative variation in
8 symbiotic nitrogen-fixation. Plant Soil 82, 329-335.

9 Mytton, L.R., Elsherbeen, M.H., Lawes, D.A., 1977. Symbiotic variability in *Vicia*
10 *faba*. 3. Genetic effects of host plant, *Rhizobium* strain and of host X strain interaction.
11 Euphytica 26, 785-791.

12 Nam, Y.W., Penmetsa, R.V., Endre, G., Uribe, P., Kim, D., Cook, D.R., 1999.
13 Construction of a bacterial artificial chromosome library of *Medicago truncatula* and
14 identification of clones containing ethylene-response genes. Theor. Appl. Genet. 98,
15 638-646.

16 Naya, L., Ladrera, R., Ramos, J., González, E.M., Arrese-Igor, C., Minchin, F.R.,
17 Becana, M., 2007. The response of carbon metabolism and antioxidant defenses of
18 alfalfa nodules to drought stress and to the subsequent recovery of plants. Plant Physiol.
19 144, 1104-1114.

20 Nelson, M.N., Phan, H.T., Ellwood, S.R., Moolhuijzen, P.M., Hane, J., Williams, A.,
21 O'Lone, C.E., Fosu-Nyarko, J., Scobie, M., Cakir, M., Jones, M.G., Bellgard, M.,
22 Ksiazkiewicz, M., Wolko, B., Barker, S.J., Oliver, R.P., Cowling, W.A., 2006. The first
23 gene-based map of *Lupinus angustifolius* L.: Location of domestication genes and
24 conserved synteny with *Medicago truncatula*. Theor. Appl. Genet. 113, 225-238.

1 Nishimura, R., Hayashi, M., Wu, G.J., Kouchi, H., Imaizumi-Anraku, H., Murakami,
2 Y., Kawasaki, S., Akao, S., Ohmori, M., Nagasawa, M., Harada, K., Kawaguchi, M.,
3 2002a. HAR1 mediates systemic regulation of symbiotic organ development. *Nature*
4 420, 426-429.

5 Nishimura, R., Ohmori, M., Fujita, H., Kawaguchi, M., 2002b. A *Lotus* basic leucine
6 zipper protein with a RING-finger motif negatively regulates the developmental
7 program of nodulation. *Proc. Natl. Acad. Sci. USA* 99, 15206-15210.

8 Nunes, C., Araujo, S.D., da Silva, J.M., Fevereiro, M.P.S., da Silva, A.B., 2008.
9 Physiological responses of the legume model *Medicago truncatula* cv. Jemalong to
10 water deficit. *Environ. Exp. Bot.* 63, 289-296.

11 Nyamsuren, O., Colditz, F., Rosendahl, S., Tamasloukht, M., Bekel, T., Meyer, F.,
12 Küster, H., Franken, P., Krajinski, F., 2003. Transcriptional profiling of *Medicago*
13 *truncatula* roots after infection with *Aphanomyces euteiches* (oomycota) identifies novel
14 genes upregulated during this pathogenic interaction. *Physiol. Mol. Plant Pathol.* 63, 17-
15 26.

16 O'Neill, N.R., Bauchan, G.R., 2000. Sources of resistance to anthracnose in the annual
17 *Medicago* core collection. *Plant Disease* 84, 261-267.

18 O'Neill, N.R., Bauchan, G.R., Samac, D.A., 2003. Reactions in the annual *Medicago*
19 *spp.* core germ plasm collection to *Phoma medicaginis*. *Plant Disease* 87, 557-562.

20 Oka-Kira, E., Tateno, K., Miura, K., Haga, T., Hayashi, M., Harada, K., Sato, S.,
21 Tabata, S., Shikazono, N., Tanaka, A., Watanabe, Y., Fukuhara, I., Nagata, T.,
22 Kawaguchi, M., 2005. Klavier (klv), a novel hypernodulation mutant of *Lotus japonicus*
23 affected in vascular tissue organization and floral induction. *Plant J.* 44, 505-515.

24 Oldroyd, G.E.D., 2007. Nodules and hormones. *Science* 315, 52-53.

1 Olsson, M., Nilsson, K., Liljenberg, C., Hendry, G.A.F., 1996. Drought stress in
2 seedlings: Lipid metabolism and lipid peroxidation during recovery from drought in
3 *Lotus corniculatus* and *Cerastium fontanum*. *Physiol. Plant.* 96, 577-584.

4 Paterson, A.H., Bowers, J.E., Burow, M.D., Draye, X., Elsik, C.G., Jiang, C.X., Katsar,
5 C.S., Lan, T.H., Lin, Y.R., Ming, R., Wright, R.J., 2000. Comparative genomics of
6 plant chromosomes. *Plant Cell* 12, 1523-1540.

7 Pedrosa, A., Sandal, N., Stougaard, J., Schweizer, D., Bachmair, A., 2002.
8 Chromosomal map of the model legume *Lotus japonicus*. *Genetics* 161, 1661-1672.

9 Penmetsa, R.V., Cook, D.R., 1997. A legume ethylene-insensitive mutant hyperinfected
10 by its rhizobial symbiont. *Science* 275, 527-530.

11 Penmetsa, R.V., Cook, D.R., 2000. Production and characterization of diverse
12 developmental mutants of *Medicago truncatula*. *Plant Physiol.* 123, 1387-1398.

13 Penmetsa, R.V., Uribe, P., Anderson, J., Lichtenzweig, J., Gish, J.C., Nam, Y.W.,
14 Engstrom, E., Xu, K., Sckisel, G., Pereira, M., Baek, J.M., López-Meyer, M., Long,
15 S.R., Harrison, M.J., Singh, K.B., Kiss, G.B., Cook, D.R., 2008. The *Medicago*
16 *truncatula* ortholog of Arabidopsis EIN2, sickle, is a negative regulator of symbiotic
17 and pathogenic microbial associations. *Plant J.* 55, 580-595.

18 Perlick, A.M., Pühler, A., 1993. A survey of transcripts expressed specifically in root
19 nodules of broadbean (*Vicia faba* L). *Plant Mol. Biol.* 22, 957-970.

20 Perry, J.A., Wang, T.L., Welham, T.J., Gardner, S., Pike, J.M., Yoshida, S., Parniske,
21 M., 2003. A TILLING reverse genetic tool and a web-accessible collection of mutants
22 of the legume *Lotus japonicus*. *Plant Physiol.* 131, 866-871.

23 Phan, H.T.T., Ellwood, S.R., Ford, R., Thomas, S., Oliver, R., 2006. Differences in
24 syntenic complexity between *Medicago truncatula* with *Lens culinaris* and *Lupinus*
25 *albus*. *Funct. Plant Biol.* 33, 775-782.

1 Phan, H.T., Ellwood, S.R., Adhikari, K., Nelson, M.N., Oliver, R.P., 2007. The first
2 genetic and comparative map of white lupin (*Lupinus albus* L.): Identification of QTLs
3 for anthracnose resistance and flowering time, and a locus for alkaloid content. DNA
4 Res. 14, 59-70.

5 Phinney, B.S., Thelen, J.J., 2005. Proteomic characterization of a triton-insoluble
6 fraction from chloroplasts defines a novel group of proteins associated with
7 macromolecular structures. J. Proteome Res. 4, 497-506.

8 Pierre, J.B., Huguet, T., Barre, P., Huyghe, C., Julier, B., 2008. Detection of QTLs for
9 flowering date in three mapping populations of the model legume species *Medicago*
10 *truncatula*. Theor. Appl. Genet. 117, 609-620.

11 Pilet-Nayel, M.L., Muehlbauer, F.J., McGee, R.J., Kraft, J.M., Baranger, A., Coyne,
12 C.J., 2005. Consistent quantitative trait loci in pea for partial resistance to *Aphanomyces*
13 *euteiches* isolates from the United States and France. Phytopathology 95, 1287-1293.

14 Plowright, R.A., Caubel, G., Mizen, K.A., 2002. *Ditylenchus* species. In: Starr, J.L.,
15 Cook, R., Bridge, J. (Eds.), Plant resistance to parasitic nematodes. CABI publishing,
16 pp. 107-139.

17 Poch, H.L., López, R.H., Clark, S.J., 2007. Ecotypes of the model legume *Lotus*
18 *japonicus* vary in their interaction phenotypes with the root-knot nematode
19 *Meloidogyne incognita*. Ann. Bot. 99, 1223-1229.

20 Postma, J.G., Jacobsen, E., Feenstra, W.J., 1988. Three pea mutants with an altered
21 nodulation studied by genetic analysis and grafting. J. Plant Physiol. 132, 424-430.

22 Poulsen, M.H., 1977. Obligate autogamy in *Vicia faba* L. J. Agric. Sci. (Camb.) 88,
23 253-256.

24 Prats, E., Llamas, M.J., Rubiales, D., 2007. Characterization of resistance mechanisms
25 to *Erysiphe pisi* in *Medicago truncatula*. Phytopathology 97, 1049-1053.

1 Prioul-Gervais, S., Deniot, G., Receveur, E.M., Frankewitz, A., Fourmann, M., Rameau,
2 C., Pilet-Nayel, M.L., Baranger, A., 2007. Candidate genes for quantitative resistance to
3 *Mycosphaerella pinodes* in pea (*Pisum sativum* L.). Theor. Appl. Genet. 114, 971-984.

4 Radutoiu, S., Madsen, L.H., Madsen, E.B., Felle, H.H., Umehara, Y., Gronlund, M.,
5 Sato, S., Nakamura, Y., Tabata, S., Sandal, N., Stougaard, J., 2003. Plant recognition of
6 symbiotic bacteria requires two LysM receptor-like kinases. Nature 425, 585-592.

7 Rech, E.L., Vianna, G.R., Aragao, F.J., 2008. High-efficiency transformation by
8 biolistics of soybean, common bean and cotton transgenic plants. Nat. Protoc. 3, 410-
9 418.

10 Repetto, O., Rogniaux, H., Firnhaber, C., Zuber, H., Küster, H., Larre, C., Thompson,
11 R., Gallardo, K., 2008. Exploring the nuclear proteome of *Medicago truncatula* at the
12 switch towards seed filling. Plant J. 56, 398-410.

13 Ricoult, C., Cliquet, J.B., Limami, A.M., 2005. Stimulation of alanine amino transferase
14 (AlaAT) gene expression and alanine accumulation in embryo axis of the model legume
15 *Medicago truncatula* contribute to anoxia stress tolerance. Physiol. Plant. 123, 30-39.

16 Ricoult, C., Echeverria, L.O., Cliquet, J.B., Limami, A.M., 2006. Characterization of
17 alanine aminotransferase (AlaAT) multigene family and hypoxic response in young
18 seedlings of the model legume *Medicago truncatula*. J. Exp. Bot. 57, 3079-3089.

19 Rodriguez-Conde, M.F., Moreno, M.T., Cubero, J.I., Rubiales, D., 2004.
20 Characterization of the *Orobanche-Medicago truncatula* association for studying early
21 stages of the parasite-host interaction. Weed Res. 44, 218-223.

22 Román, B., Torres, A.M., Rubiales, D., Cubero, J.I., Satovic, Z., 2002. Mapping of
23 quantitative trait loci controlling broomrape (*Orobanche crenata* Forsk.) resistance in
24 faba bean (*Vicia faba* L.). Genome 45, 1057-1063.

1 Román, B., Satovic, Z., Ávila, C.M., Rubiales, D., Moreno, M.T., Torres, A.M., 2003.
2 Locating genes associated with *Ascochyta fabae* resistance in *Vicia faba*. Austr. J.
3 Agric. Res. 54, 85-90.

4 Román, B., Satovic, Z., Pozarkova, D., Macas, J., Dolezel, J., Cubero, J.I., Torres,
5 A.M., 2004. Development of a composite map in *Vicia faba*, breeding applications and
6 future prospects. Theor. Appl. Genet. 108, 1079-1088.

7 Rose, R.J., 2008. *Medicago truncatula* as a model for understanding plant interactions
8 with other organisms, plant development and stress biology: Past, present and future.
9 Funct. Plant Biol. 35, 253-264.

10 Rubiales, D., Moral, A., 2004. Prehaustorial resistance against alfalfa rust (*Uromyces*
11 *striatus*) in *Medicago truncatula*. Eur. J. Plant Pathol. 110, 239-243.

12 Rubio, M.C., González, E.M., Minchin, F.R., Webb, K.J., Arrese-Igor, C., Ramos, J.,
13 Becana, M., 2002. Effects of water stress on antioxidant enzymes of leaves and nodules
14 of transgenic alfalfa overexpressing superoxide dismutases. Physiol. Plant. 115, 531-
15 540.

16 Saito, K., Yoshikawa, M., Yano, K., Miwa, H., Uchida, H., Asamizu, E., Sato, S.,
17 Tabata, S., Imaizumi-Anraku, H., Umehara, Y., Kouchi, H., Murooka, Y.,
18 Szczyglowski, K., Downie, J.A., Parniske, M., Hayashi, M., Kawaguchi, M., 2007.
19 NUCLEOPORIN85 is required for calcium spiking, fungal and bacterial symbioses, and
20 seed production in *Lotus japonicus*. Plant Cell 19, 610-624.

21 Sanchez, D.H., Lippold, F., Redestig, H., Hannah, M.A., Erban, A., Kramer, U., Kopka,
22 J., Udvardi, M.K., 2008. Integrative functional genomics of salt acclimatization in the
23 model legume *Lotus japonicus*. Plant J. 53, 973-987.

24 Sandal, N., Petersen, T.R., Murray, J., Umehara, Y., Karas, B., Yano, K., Kumagai, H.,
25 Yoshikawa, M., Saito, K., Hayashi, M., Murakami, Y., Wang, X., Hakoyama, T.,

1 Imaizumi-Anraku, H., Sato, S., Kato, T., Chen, W., Hossain, M.S., Shibata, S., Wang,
2 T., Yokota, K., Larsen, K., Kanamori, N., Madsen, E., Radutoiu, S., Madsen, L.H.,
3 Radu, T.G., Krusell, L., Ooki, Y., Banba, M., Betti, M., Rispail, N., Skøt, L., Tuck, E.,
4 Perry, J., Yoshida, S., Vickers, K., Pike, J., Mulder, L., Charpentier, M., Müller, J.,
5 Ohtomi, R., Kojima, T., Ando, S., Márquez, A.J., Gresshoff, P.M., Harada, K., Webb,
6 K.J., Hata, S., Suganuma, N., Kouchi, H., Kawasaki, S., Tabata, S., Hayashi, M.,
7 Parniske, M., Szczyglowski, K., Kawaguchi, M., Stougaard, J., 2006. Genetics of
8 symbiosis in *Lotus japonicus*: Recombinant inbred lines, comparative genetic maps and
9 map position of 36 symbiotic loci. *Mol. Plant Microbe Interac.* 19, 80-91.

10 Sato, S., Nakamura, Y., Asamizu, E., Isobe, S., Tabata, S., 2007. Genome sequencing
11 and genome resources in model legumes. *Plant Physiol.* 144, 588-593.

12 Sato, S., Nakamura, Y., Kaneko, T., Asamizu, E., Kato, T., Nakao, M., Sasamoto, S.,
13 Watanabe, A., Ono, A., Kawashima, K., Fujishiro, T., Katoh, M., Kohara, M., Kishida,
14 Y., Minami, C., Nakayama, S., Nakazaki, N., Shimizu, Y., Shinpo, S., Takahashi, C.,
15 Wada, T., Yamada, M., Ohmido, N., Hayashi, M., Fukui, K., Baba, T., Nakamichi, T.,
16 Mori, H., Tabata, S., 2008. Genome structure of the legume *Lotus japonicus*. *DNA Res.*
17 15, 227-239.

18 Schauser, L., Roussis, A., Stiller, J., Stougaard, J., 1999. A plant regulator controlling
19 development of symbiotic root nodules. *Nature* 402, 191-195.

20 Schiltz, S., Gallardo, K., Huart, M., Negroni, L., Sommerer, N., Burstin, J., 2004.
21 Proteome reference maps of vegetative tissues in pea. An investigation of nitrogen
22 mobilization from leaves during seed filling. *Plant Physiol.* 135, 2241-2260.

23 Schnabel, E., Journet, E.P., de Carvalho-Niebel, F., Duc, G., Frugoli, J., 2005. The
24 *Medicago truncatula* SUNN gene encodes a CLV1-like leucine-rich repeat receptor
25 kinase that regulates nodule number and root length. *Plant Mol. Biol.* 58, 809-822.

1 Schröder, G., Frühling, M., Pühler, A., Perlick, A.M., 1997. The temporal and spatial
2 transcription pattern in root nodules of *Vicia faba* nodulin genes encoding glycine-rich
3 proteins. Plant Mol. Biol. 33, 113-123.

4 Schumpp, O., Ramel, M.E., Gugerli, P., Broughton, W.J., Deakin, W.J., 2007.
5 Identification of a *Lotus* viral pathogen. J. Plant Res. 120, 651-654.

6 Searle, I.R., Men, A.E., Laniya, T.S., Buzás, D.M., Iturbe-Ormaetxe, I., Carroll, B.J.,
7 Gresshoff, P.M., 2003. Long-distance signaling in nodulation directed by a
8 CLAVATA1-like receptor kinase. Science 299, 109-112.

9 Shimoda, Y., Nagata, M., Suzuki, A., Abe, M., Sato, S., Kato, T., Tabata, S., Higashi,
10 S., Uchiumi, T., 2005. Symbiotic rhizobium and nitric oxide induce gene expression of
11 non-symbiotic hemoglobin in *Lotus japonicus*. Plant Cell Physiol. 46, 99-107.

12 Shimoda, Y., Shimoda-Sasakura, F., Kucho, K.I., Kanamori, N., Nagata, M., Suzuki,
13 A., Abe, M., Higashi, S., Uchiumi, T., 2008. Overexpression of class 1 plant
14 hemoglobin genes enhances symbiotic nitrogen fixation activity between
15 *Mesorhizobium loti* and *Lotus japonicus*. Plant J. 57, 254-263.

16 Simon, C.J., Muehlbauer, F.J., 1997. Construction of a chickpea linkage map and its
17 comparison with maps of pea and lentil. J. Heredity 88, 115-119.

18 Smit, P., Raedts, J., Portyanko, V., Debellé, F., Gough, C., Bisseling, T., Geurts, R.,
19 2005. NSP1 of the GRAS protein family is essential for rhizobial Nod factor-induced
20 transcription. Science 308, 1789-1791.

21 Stacey, G., Libault, M., Brechenmacher, L., Wan, J., May, G.D., 2006. Genetics and
22 functional genomics of legume nodulation. Curr. Opin. Plant Biol. 9, 110-121.

23 Stracke, S., Kistner, C., Yoshida, S., Mulder, L., Sato, S., Kaneko, T., Tabata, S.,
24 Sandal, N., Stougaard, J., Szczyglowski, K., Parniske, M., 2002. A plant receptor-like
25 kinase required for both bacterial and fungal symbiosis. Nature 417, 959-962.

1 Stracke, S., Sato, S., Sandal, N., Koyama, M., Kaneko, T., Tabata, S., Parniske, M.,
2 2004. Exploitation of colinear relationships between the genomes of *Lotus japonicus*,
3 *Pisum sativum* and *Arabidopsis thaliana*, for positional cloning of a legume symbiosis
4 gene. Theor. Appl. Genet. 108, 442-449.

5 Subramanian, S., Graham, M.Y., Yu, O., Graham, T.L., 2005. RNA interference of
6 soybean isoflavone synthase genes leads to silencing in tissues distal to the
7 transformation site and to enhanced susceptibility to *Phytophthora sojae*. Plant Physiol.
8 137, 1345-1353.

9 Swindell, W.R., Huebner, M., Weber, A.P., 2007. Transcriptional profiling of
10 Arabidopsis heat shock proteins and transcription factors reveals extensive overlap
11 between heat and non-heat stress response pathways. BMC Genomics 8.

12 Tadege, M., Wen, J., He, J., Tu, H., Kwak, Y., Eschstruth, A., Cayrel, A., Endre, G.,
13 Zhao, P.X., Chabaud, M., Ratet, P., Mysore, K.S., 2008. Large-scale insertional
14 mutagenesis using the *Tnt1* retrotransposon in the model legume *Medicago truncatula*.
15 Plant J. 54, 335-347.

16 Tar'an, B., Warkentin, T., Somers, D.J., Miranda, D., Vandenburg, A., Blade, S.,
17 Woods, S., Bing, D., Xue, A., DeKoeyer, D., Penner, G., 2003. Quantitative trait loci
18 for lodging resistance, plant height and partial resistance to mycosphaerella blight in
19 field pea (*Pisum sativum* L.). Theor. Appl. Genet. 107, 1482-1491.

20 Tar'an, B., Warkentin, T., Somers, D.J., Miranda, D., Vandenberg, A., Blade, S., Bing,
21 D., 2004. Identification of quantitative trait loci for grain yield, seed protein
22 concentration and maturity in field pea (*Pisum sativum* L.). Euphytica 136, 297-306.

23 Tivoli, B., Baranger, A., Sivasithamparam, K., Barbetti, M.J., 2006. Annual *Medicago*:
24 From a model crop challenged by a spectrum of necrotrophic pathogens to a model
25 plant to explore the nature of disease resistance. Ann. Bot. 98, 1117-1128.

1 Thomashow, M.F., 1999. Plant cold acclimation: Freezing tolerance genes and
2 regulatory mechanisms. *Annu. Rev. Plant Phys.* 50, 571-599.

3 Thompson, R., Ratet, P., Küster, H., 2005. Identification of gene functions by applying
4 TILLING and insertional mutagenesis strategies on microarray-based expression data.
5 *Grain Legumes Magazine*, pp. 20-22.

6 Thoquet, P., Ghérardi, M., Journet, E.P., Kereszt, A., Ané, J.M., Prosperi, J.M., Huguet,
7 T., 2002. The molecular genetic linkage map of the model legume *Medicago truncatula*:
8 an essential tool for comparative legume genomics and the isolation of agronomically
9 important genes. *BMC Plant Biol.* 2, 1.

10 Thykjaer, T., Stiller, J., Handberg, K., Jones, J., Stougaard, J., 1995. The maize
11 transposable element *Ac* is mobile in the legume *Lotus japonicus*. *Plant Mol. Biol.* 27,
12 981-993.

13 Timmerman-Vaughan, G.M., Mills, A., Whitfield, C., Frew, T., Butler, R., Murray, S.,
14 Lakeman, M., McCallum, J., Russell, A., Wilson, D., 2005. Linkage mapping of QTL
15 for seed yield, yield components, and developmental traits in pea. *Crop Sci.* 45, 1336-
16 1344.

17 Tirichine, L., Sandal, N., Madsen, L.H., Radutoiu, S., Albrektsen, A.S., Sato, S.,
18 Asamizu, E., Tabata, S., Stougaard, J., 2007. A gain-of-function mutation in a cytokinin
19 receptor triggers spontaneous root nodule organogenesis. *Science* 315, 104-107.

20 Torregrosa, C., Cluzet, S., Fournier, J., Huguet, T., Gamas, P., Prosperi, J.M., Esquerre-
21 Tugaye, M.T., Dumas, B., Jacquet, C., 2004. Cytological, genetic, and molecular
22 analysis to characterize compatible and incompatible interactions between *Medicago*
23 *truncatula* and *Colletotrichum trifolii*. *Mol. Plant Microbe Interac.* 17, 909-920.

1 Tougou, M., Yamagishi, N., Furutani, N., Shizukawa, Y., Takahata, Y., Hidaka, S.,
2 2007. Soybean dwarf virus-resistant transgenic soybeans with the sense coat protein
3 gene. *Plant Cell Rep.* 26, 1967-1975.

4 Tramontano, W.A., Jouve, D., 1997. Trigonelline accumulation in salt-stressed legumes
5 and the role of other osmoregulators as cell cycle control agents. *Phytochemistry* 44,
6 1037-1040.

7 Trinchant, J.C., Boscari, A., Spermat, G., Van de Sype, G., Le Rudulier, D., 2004.
8 Proline betaine accumulation and metabolism in alfalfa plants under sodium chloride
9 stress. Exploring its compartmentalization in nodules. *Plant Physiol.* 135, 1583-1594.

10 Vailleau, F., Sartorel, E., Jardinaud, M.F., Chardon, F., Genin, S., Huguet, T.,
11 Gentzbittel, L., Petitprez, M., 2007. Characterization of the interaction between the
12 bacterial wilt pathogen *Ralstonia solanacearum* and the model legume plant *Medicago*
13 *truncatula*. *Mol. Plant Microbe Interac.* 20, 159-167.

14 Valot, B., Negroni, L., Zivy, M., Gianinazzi, S., Dumas-Gaudot, E., 2006. A mass
15 spectrometric approach to identify arbuscular mycorrhiza-related proteins in root
16 plasma membrane fractions. *Proteomics* 6 Suppl. 1, S145-155.

17 van Hengel, A.J., Barber, C., Roberts, K., 2004. The expression patterns of
18 arabinogalactan-protein AtAGP30 and GLABRA2 reveal a role for abscisic acid in the
19 early stages of root epidermal patterning. *Plant J.* 39, 70-83.

20 van Noorden, G.E., Kerim, T., Goffard, N., Wiblin, R., Pellerone, F.I., Rolfe, B.G.,
21 Mathesius, U., 2007. Overlap of proteome changes in *Medicago truncatula* in response
22 to auxin and *Sinorhizobium meliloti*. *Plant Physiol.* 144, 1115-1131.

23 Vavilov, N., 1922. The law of homologous series in variation. *J. Genet.* 12, 47-88.

1 Vaz Patto, M.C., Torres, A.M., Koblizkova, A., Macas, J., Cubero, J.I., 1999.
2 Development of a genetic composite map of *Vicia faba* using F-2 populations derived
3 from trisomic plants. Theor. Appl. Genet. 98, 736-743.

4 Verdier, J., Kakar, K., Gallardo, K., Le Signor, C., Aubert, G., Schlereth, A., Town,
5 C.D., Udvardi, M.K., Thompson, R.D., 2008. Gene expression profiling of *M.*
6 *truncatula* transcription factors identifies putative regulators of grain legume seed
7 filling. Plant Mol. Biol. 67, 567-580.

8 Verdoy, D., Coba De La Pena, T., Redondo, F.J., Lucas, M.M., Pueyo, J.J., 2006.
9 Transgenic *Medicago truncatula* plants that accumulate proline display nitrogen-fixing
10 activity with enhanced tolerance to osmotic stress. Plant Cell Environ. 29, 1913-1923.

11 Vernié, T., Moreau, S., de Billy, F., Plet, J., Combier, J.P., Rogers, C., Oldroyd, G.,
12 Frugier, F., Niebel, A., Gamas, P., 2008. EFD is an ERF transcription factor involved in
13 the control of nodule number and differentiation in *Medicago truncatula*. Plant Cell 20,
14 2693-2713.

15 Vieweg, M.F., Frühling, M., Quandt, H.J., Heim, U., Bäumlein, H., Pühler, A., Küster,
16 H., Andreas, M.P., 2004. The promoter of the *Vicia faba* L. leghemoglobin gene
17 VfLb29 is specifically activated in the infected cells of root nodules and in the
18 arbuscule-containing cells of mycorrhizal roots from different legume and non legume
19 plants. Mol. Plant Microbe Interac. 17, 62-69.

20 Wang, X., Sato, S., Tabata, S., Kawasaki, S., 2008. A high-density linkage map of *Lotus*
21 *japonicus* based on AFLP and SSR markers. DNA Res. 15, 323-332.

22 Watson, B.S., Asirvatham, V.S., Wang, L., Sumner, L.W., 2003. Mapping the proteome
23 of barrel medic (*Medicago truncatula*). Plant Physiol. 131, 1104-1123.

24 Webb, K.J., Skøt, L., Nicholson, M.N., Jorgensen, B., Mizen, S., 2000. *Mesorhizobium*
25 *loti* increases root-specific expression of a calcium-binding protein homologue

1 identified by promoter tagging in *Lotus japonicus*. Mol. Plant Microbe Interac. 13, 606-
2 616.

3 Weeden, N.F., Muehlbauer, F.J., Ladizinsky, G., 1992. Extensive conservation of
4 linkage relationships between pea and lentil genetic maps. J. Heredity 83, 123-129.

5 Weeden, N.F., Ellis, T.H.N., Timmerman-Vaughan, G.M., Swiecicki, W.K., Rozov,
6 S.M., Berdnikov, V.A., 1998. A consensus linkage map for *Pisum sativum*. Pisum
7 Genetic 30, 1-4.

8 Weerasinghe, R.R., Bird, D.M., Allen, N.S., 2005. Root-knot nematodes and bacterial
9 Nod factors elicit common signal transduction events in *Lotus japonicus*. Proc. Natl.
10 Acad. Sci. USA 102, 3147-3152.

11 Weigelt, K., Küster, H., Radchuk, R., Müller, M., Weichert, H., Fait, A., Fernie, A.R.,
12 Saalbach, I., Weber, H., 2008. Increasing amino acid supply in pea embryos reveals
13 specific interactions of N and C metabolism, and highlights the importance of
14 mitochondrial metabolism. Plant J. 55, 909-926.

15 Wesley, S.V., Helliwell, C.A., Smith, N.A., Wang, M.B., Rouse, D.T., Liu, Q.,
16 Gooding, P.S., Singh, S.P., Abbott, D., Stoutjesdijk, P.A., Robinson, S.P., Gleave, A.P.,
17 Green, A.G., Waterhouse, P.M., 2001. Construct design for efficient, effective and high-
18 throughput gene silencing in plants. Plant J. 27, 581-590.

19 Wienkoop, S., Saalbach, G., 2003. Proteome analysis. Novel proteins identified at the
20 peribacteroid membrane from *Lotus japonicus* root nodules. Plant Physiol. 131, 1080-
21 1090.

22 Winicov, I., Bastola, D.R., 1999. Transgenic overexpression of the transcription factor
23 *Alfin1* enhances expression of the endogenous *MsPRP2* gene in alfalfa and improves
24 salinity tolerance of the plants. Plant Physiol. 120, 473-480.

1 Wu, F., Mueller, L.A., Crouzillat, D., Petiard, V., Tanksley, S.D., 2006. Combining
2 bioinformatics and phylogenetics to identify large sets of single-copy orthologous genes
3 (COSII) for comparative, evolutionary and systematic studies: A test case in the
4 euasterid plant clade. *Genetics* 174, 1407-1420.

5 Xu, Y., Ma, Q.H., 2004. *Medicago truncatula* Mt-ZFP1 encoding a root enhanced zinc
6 finger protein is regulated by cytokinin, abscisic acid and jasmonate, but not cold. *DNA*
7 *Seq.* 15, 104-109.

8 Yaege, J.R., Stuteville, D.L., 2000. Reactions in the annual *Medicago* core germ plasm
9 collection to two isolates of *Peronospora trifoliorum* from alfalfa. *Plant Disease* 84,
10 521-524.

11 Yamada, K., Shimada, T., Kondo, M., Nishimura, M., Hara-Nishimura, I., 1999.
12 Multiple functional proteins are produced by cleaving Asn-Gln bonds of a single
13 precursor by vacuolar processing enzyme. *J. Biol. Chem.* 274, 2563-2570.

14 Yamaguchi, T., Blumwald, E., 2005. Developing salt-tolerant crop plants: Challenges
15 and opportunities. *Trends Plant Sci.* 10, 615-620.

16 Yang, S., Gao, M., Deshpande, S., Lin, S., Roe, B.A., Zhu, H., 2007. Genetic and
17 physical localization of an anthracnose resistance gene in *Medicago truncatula*. *Theor.*
18 *Appl. Genet.* 116, 45-52.

19 Yang, S., Gao, M., Xu, C., Gao, J., Deshpande, S., Lin, S., Roe, B.A., Zhu, H., 2008.
20 Alfalfa benefits from *Medicago truncatula*: The RCT1 gene from *M. truncatula* confers
21 broad-spectrum resistance to anthracnose in alfalfa. *Proc. Natl. Acad. Sci. USA* 105,
22 12164-12169.

23 Zhang, C.Q., Ghabrial, S.A., 2006. Development of bean pod mottle virus-based vectors
24 for stable protein expression and sequence-specific virus-induced gene silencing in
25 soybean. *Virology* 344, 401-411.

- 1 Zhang, J.Y., Broeckling, C.D., Blancaflor, E.B., Sledge, M.K., Sumner, L.W., Wang,
2 Z.Y., 2005. Overexpression of WXP1, a putative *Medicago truncatula* AP2 domain-
3 containing transcription factor gene, increases cuticular wax accumulation and enhances
4 drought tolerance in transgenic alfalfa (*Medicago sativa*). Plant J. 42, 689-707.
- 5 Zhu, H., Choi, H.K., Cook, D.R., Shoemaker, R.C., 2005. Bridging model and crop
6 legumes through comparative genomics. Plant Physiol. 137, 1189-1196.

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Tables

Table 1. Comparison of genome size, seed composition and available resources between *Vicia faba*, *Pisum sativum* and *Medicago truncatula*. Phylogeny for the species according to Choi *et al.* (2004a) is represented as a tree on the left.

Species	Genome size (Mb)	Genomic resources * NCBI, release 31 July 2008	Genetic resources	QTL mapping	Post-genomic resources *NCBI, release 31 July 2008	Seed composition
<i>Vicia faba</i> Tribe <i>Viceae</i>	~13,000 (2n = 12 chrom.)	466 core nucleotide sequences * 512 genome survey sequences*	Mapping populations and linkage maps: <ul style="list-style-type: none"> ~20 F2 populations 14 major linkage groups Vaz Patto <i>et al.</i> 1999; Ávila <i>et al.</i> 2003, 2004; Román <i>et al.</i> 2004 and references therein <i>Vicia faba</i> germplasm collections at: <ul style="list-style-type: none"> http://193.50.15.18/legumbase/ http://www.icarda.cgiar.org/ 	QTL mapped for seed weight and disease resistance Vaz Patto <i>et al.</i> 1999; Ávila <i>et al.</i> 2004; Román <i>et al.</i> 2002, 2003	<10 EST*	30% proteins 42% starch 2% lipids 9% cellulose 4% soluble sugars (Duc <i>et al.</i> , 1999)
<i>Pisum sativum</i> Tribe <i>Viceae</i>	~5,000 (2n=14 chrom.)	3,864 core nucleotide sequences* 154 genome survey sequences* BAC libraries: <ul style="list-style-type: none"> Line PI269818: ~3 genome equivalents (Coyne <i>et al.</i>, 2007) Cv. Caméor: ~10 genome equivalents (A. Bendahmane, personal communication) 	Mapping populations and linkage maps: <ul style="list-style-type: none"> ~20 population (F2, F5 to F9) 7 linkage groups (Weeden <i>et al.</i> , 1998 ; Ellis and Poyser 2002 ; Tar'an <i>et al.</i> , 2003; Loridon <i>et al.</i> , 2005 ; Pilet-Nayel <i>et al.</i> , 2005 ; Aubert <i>et al.</i> , 2006) Pea germplasm collections: <ul style="list-style-type: none"> http://193.50.15.18/legumbase/ http://www.jic.ac.uk/germplas/pisum/; www.ars-grin.gov/ Mutant populations: TILLING (Dalmais <i>et al.</i> , 2008)	QTL mapped for a range of agronomic traits e.g. seed traits, disease resistance, frost tolerance (Tar'an <i>et al.</i> , 2004; Pilet-Nayel <i>et al.</i> , 2005; Timmermann-Vaughan <i>et al.</i> , 2005; Burstin <i>et al.</i> , 2007; Prioul-Gervais <i>et al.</i> , 2007; Lejeune-Hénaut <i>et al.</i> , 2008)	EST count : 6,327 ESTs* (580 from seed cDNA libraries) Transcriptomics : Ps6kOLI1 microarrays (Weigelt <i>et al.</i> , 2008) Proteome maps : e.g. leaves (Schiltz <i>et al.</i> , 2004), mature seeds (Bourgeois <i>et al.</i> , 2008), and organelles (Bardel <i>et al.</i> , 2002 ; Phinney and Thelen 2005)	23% proteins 50% starch 2% lipids 7% cellulose 5% soluble sugars (Bastianelli <i>et al.</i> , 1998)
<i>Medicago truncatula</i> Tribe <i>Trifolieae</i>	~500 (2n=16 chrom.)	6,149 core nucleotide sequences * 168,815 genome survey sequences* BAC libraries: 5 to 20 genome equivalents (Nam <i>et al.</i> , 1999; Choi <i>et al.</i> , 2004a) Sequencing gene-rich regions http://www.medicago.org/genome/	Mapping populations and linkage maps: <ul style="list-style-type: none"> ~800 populations, including five F6 to F7 populations of recombinant inbred lines 8 linkage groups (Thoquet <i>et al.</i> , 2002 ; Green <i>et al.</i> , 2006) <i>M. truncatula</i> germplasm collections: (Greene <i>et al.</i> , 2006) Mutant populations: <ul style="list-style-type: none"> TILLING (Thompson <i>et al.</i> 2005), <i>Tnt1</i> insertion (Tadege <i>et al.</i> 2008) 	QTL mapped for aerial morphogenesis, including flowering, resistance to disease, spring black stem and leaf spot (Julier <i>et al.</i> , 2007 ; Vailleau <i>et al.</i> , 2007 ; Ameline-Torregrosa <i>et al.</i> , 2008; Kamphuis <i>et al.</i> , 2008 ; Pierre <i>et al.</i> , 2008)	EST count: 249,625 ESTs* (12,937 from seed cDNA libraries) Transcriptomics : Mt16kOLI1 microarrays (Hohnjec <i>et al.</i> , 2005) Affymetrix Medicago GeneChip (Gene expression atlas from Bénédicto <i>et al.</i> , 2008) Proteome maps from various tissues, including seeds: (Watson <i>et al.</i> , 2003)	46% proteins 1% starch 13% lipids 10% cellulose 5% soluble sugars (Duc, 2004)

Figure legends

Figure 1. Large-scale synteny blocks between *M. truncatula* (Mt) and *L. japonicus* (Lj) chromosomes. Synteny was detected along entire chromosomal segments in the case of *M. truncatula* chr 1 and *L. japonicus* chr 5, while rearrangements of colinear blocks in other chromosomal regions resulted in a difference of chromosome number between the two species. Bars with the same color or pattern show homologous chromosomal regions and arrows in the boxes indicate the orientation of the chromosomes (short arm – long arm). Blank boxes represent genomic regions where the correspondence between the chromosomes has not yet been revealed clearly.

Figure 2. Signaling of nodulation. **A.** Nodulation factor perception leads to infection and cell division in legume roots. NF signal transduction occurs through four biochemical stages, beginning with receptor kinases, then membrane channels causing calcium oscillations, followed by a protein kinase modulator (CCaMK) and a cytokinin receptor, and finally through transcription factor control of diverse genetic pathways. This cascade occurs in different cell layers of the root. For example, NF perception likely occurs primarily in the root hair and the epidermis, perhaps fractionally so in the cortex. Note that epidermal and root hair cells do not divide and hence there is a need for a secondary signal to travel to the root interior and the pericycle, the target tissue for nodule growth. Later parts of the cascade involving transcription factors may be localized to this region. Detailed spatial analysis using gene fusions and reporter genes is needed to elucidate such events. **B.** Autoregulation of Nodulation (AON) controls nodule number by a systemic regulatory circuit involving root-shoot communication. NF perception leads to an activated state closely associated with the induction of cell divisions. This process may be mimicked by mycorrhizal fungi, which undergo

eukaryotic cell cycle progression similar to that of plant cells (cf., Meixner et al, 2007). This activated state is ‘reinforced’ by successful invasion and nitrogen fixation. The mobile ‘Q’ signal is perceived by leaf tissues to release a putative ligand peptide that interacts with the NARK receptor kinase in the phloem parenchyma of the leaf. NARK is known to down-regulate several genes in the octadecanoid pathway leading to the synthesis of jasmonic acid (JA). Metabolites related to this pathway or parallel targets of NARK, including ‘SDI’, are presumed to migrate via the phloem to the developing nodule primordium, blocking further nodule development. In temperate legumes, such as pea and *M. truncatula*, this blockage occurs earlier than in the determinate-nodulating soybean (Mathews et al., 1989).

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2 **Figure 1**

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1 **Figure 2**