

## Lotus literature

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BORISOV A.Y., MADSEN L.H., TSYGANOV V.E., UMEHARA Y., VOROSHILOVA V.A., BATAGOV A.O., SANDAL A. FREDERIKSEN N., SCHAUSER L., ELLIS N., TIKHONOVICH I.A. and **STOUGAARD J.** 2003. The *Sym35* gene required for root nodule development in *Pisum sativum* is an orthologue of *Nin* gene from *Lotus japonicus*. *Plant Physiology*, **131**, 1009-1017.

Comparative phenotypic analysis of pea (*Pisum sativum*) *sym35* mutants and *Lotus japonicus nin* mutants suggested a similar function for the *PsSym35* and *LjNin* genes in early stages of root nodule formation. Both the pea and *L. japonicus* mutants are non-nodulating but normal in their arbuscular mycorrhizal association. Both are characterized by excessive root hair curling in response to the bacterial microsymbiont, lack of infection thread initiation, and absence of cortical cell divisions. To investigate the molecular basis for the similarity, we cloned and sequenced the *PsNin* gene, taking advantage of sequence information from the previously cloned *LjNin* gene. An RFLP analysis on recombinant inbred lines mapped *PsNin* to the same chromosome arm as the *PsSym35* locus and direct evidence demonstrating that *PsNin* is the *PsSym35* gene was subsequently obtained by cosegregation analysis and sequencing of three independent *Pssym35* mutant alleles. *L. japonicus* and pea root nodules develop through different organogenic pathways, so it was of interest to compare the expression of the two orthologous genes during nodule formation. Overall, a similar developmental regulation of the *PsNin* and *LjNin* genes was shown by the transcriptional activation in root nodules of *L. japonicus* and pea. In the indeterminate pea nodules, *PsNin* is highly expressed in the meristematic cells of zone I and in the cells of infection zone II, corroborating expression of *LjNin* in determinate nodule primordia. At the protein level, seven domains, including the putative DNA binding/dimerization RWP-RK motif and the PB1 heterodimerization domain, are conserved between the *LjNIN* and *PsNIN* proteins.

Full text at <http://www.plantphysiol.org/cgi/content/full/131/3/1009>

BONFANTE P., GENRE A., FACCIO A., MARTINI I., SCHAUSER L., **STOUGAARD J.**, WEBB J. and PARNISKE M. 2000. The *Lotus japonicus LjSym4* gene is required for the successful symbiotic infection of root epidermal cells. *Molecular Plant Microbe Interactions*, **13**, 1109-1120.

The role of the *Lotus japonicus LjSym4* gene during the symbiotic interaction with *Mesorhizobium loti* and arbuscular mycorrhizal (AM) fungi was analyzed with two mutant alleles conferring phenotypes of different strength. *Ljsym4-1* and *Ljsym4-2* mutants do not form nodules with *M. loti*. Normal root hair curling and infection threads are not observed, while a *nodC*-dependent deformation of root hair tips indicates that nodulation factors are still perceived by *Ljsym4* mutants. Fungal infection attempts on the mutants generally abort within the epidermis, but *Ljsym4-1* mutants allow rare, successful, infection events, leading to delayed arbuscule formation. On roots of mutants homozygous for the *Ljsym4-2* allele, arbuscule formation was never observed upon inoculation with either of the two AM fungi, *Glomus intraradices* or *Gigaspora margarita*. The strategy of epidermal penetration by *G. margarita* was identical for *Ljsym4-2* mutants and the parental line, with appressoria, hyphae growing between two epidermal cells, penetration of epidermal cells through their anticlinal wall. These observations define a novel, genetically controlled step in AM colonization. Although rhizobia penetrate the tip of root hairs and AM fungi access an entry site near the base of epidermal cells, the

LjSym4 gene is necessary for the appropriate response of this cell type to both microsymbionts. We propose that LjSym4 is required for the initiation or coordinated expression of the host plant cell's accommodation program, allowing the passage of both microsymbionts through the epidermis layer.

FORSLUND K., MORANT M., JORGENSEN B., OLSEN C.E., ASAMIZU E., SATO S., TABATA S. and **BAK S.** 2004. Biosynthesis of the nitrile glucosides rhodiocyanoside A and D and the cyanogenic glucosides lotaustralin and linamarin in *Lotus japonicus*. *Plant Physiology*, **135**, 71-84.

*Lotus japonicus* was shown to contain the two nitrile glucosides rhodiocyanoside A and rhodiocyanoside D as well as the cyanogenic glucosides linamarin and lotaustralin. The content of cyanogenic and nitrile glucosides in *L. japonicus* depends on plant developmental stage and tissue. The cyanide potential is highest in young seedlings and in apical leaves of mature plants. Roots and seeds are acyanogenic. Biosynthetic studies using radioisotopes demonstrated that lotaustralin, rhodiocyanoside A, and rhodiocyanoside D are derived from the amino acid l-Ile, whereas linamarin is derived from Val. In silico homology searches identified two cytochromes P450 designated CYP79D3 and CYP79D4 in *L. japonicus*. The two cytochromes P450 are 94% identical at the amino acid level and both catalyze the conversion of Val and Ile to the corresponding aldoximes in biosynthesis of cyanogenic glucosides and nitrile glucosides in *L. japonicus*. CYP79D3 and CYP79D4 are differentially expressed. CYP79D3 is exclusively expressed in aerial parts and CYP79D4 in roots. Recombinantly expressed CYP79D3 and CYP79D4 in yeast cells showed higher catalytic efficiency with l-Ile as substrate than with l-Val, in agreement with lotaustralin and rhodiocyanoside A and D being the major cyanogenic and nitrile glucosides in *L. japonicus*. Ectopic expression of CYP79D2 from cassava (*Manihot esculenta* Crantz.) in *L. japonicus* resulted in a 5- to 20-fold increase of linamarin content, whereas the relative amounts of lotaustralin and rhodiocyanoside A/D were unaltered.

**GRANT W.F.** and MCDUGALL R.B. 1995. Registration of H401-4-4-2 birdsfoot trefoil germplasm resistant to sulfonylurea. *Crop Science*, **35**, 286-287.

GRUBER M.Y., RAY H., AUSER P., SKADHAUGE B., FALK J., THOMSEN K.K., **STOUGAARD J.**, MUIR A., LEES G., COULMAN B., MCKERSIE B., BOWLEY S. and VON WETTSTEIN D. 1999. Genetic systems for condensed tannin biotechnology. *In* GROSS G.G., HEMINGWAY R. and YOSHIDA T. (Eds.) *Plant Polyphenols 2: Chemistry and Biology*. Plenum Press, New York. [Manual]

HAYASHI M., MIYAHARA A., SATO S., KATO T., YOSHIKAWA M., TAKETA M., HAYASHI M., **PEDROSA A.**, ONDA R., IMAIZUMI-ANRAKU H., BACHMAIR A., SANDAL N., STOUGAARD J., MUROOKA Y., TABATA S., KAWASAKI S., KAWAGUCHI M. and HARADA K. 2001. Construction of a Genetic Linkage Map of the Model Legume *Lotus japonicus* Using an Intraspecific F2 Population. *DNA Research*, **8**, 301-310.

Among leguminous plants, the model legume *Lotus japonicus* (Regel) Larsen has many biological and genetic advantages. We have developed a genetic linkage map of *L. japonicus* based on amplified fragment length polymorphism (AFLP), simple sequence repeat polymorphism (SSRP) and derived cleaved amplified polymorphic sequence (dCAPS). The F2 mapping population used was derived from a cross between two *L. japonicus* accessions Gifu B-129 and Miyakojima MG-20. These parental accessions showed remarkable cytological differences, particularly with respect to size and morphology of chromosomes 1 and 2. Using fluorescence *in situ* hybridization (FISH) with BAC clones from Gifu B-129 and TAC (Transformation-competent Artificial Chromosome) clones from

Miyakojima MG-20, a reciprocal translocation was found to be responsible for the cytological differences between chromosomes 1 and 2. The borders of the translocations were identified by FISH and by alignment toward the *L. filicaulis*×*L. japonicus* Gifu B-129 linkage map. The markers from the main translocated region were located on linkage groups 1 and 2 of the two accessions, Gifu B-129 and Miyakojima MG-20, respectively. The framework of the linkage map was constructed based on codominant markers, and then dominant markers were integrated separately in each linkage group of the parents. The resulting linkage groups correspond to the six pairs of chromosomes of *L. japonicus* and consist of 287 markers with 487.3 cM length in Gifu B-129 and 277 markers with 481.6 cM length in Miyakojima MG-20. The map and marker information is available through the World Wide Web at <http://www.kazusa.or.jp/lotus/>.

Free text at [http://dna-res.kazusa.or.jp/8/6/06/PDF/8\\_301.pdf](http://dna-res.kazusa.or.jp/8/6/06/PDF/8_301.pdf)

**KADE M., PAGANI E.A. and MENDOZA R.E.** 2003. Phosphorus utilization efficiency in populations of narrow leaf-birdsfoot trefoil. *Communications in Soil Science and Plant Analysis*, **34**, 271-284.

The widespread distribution of the pasture legume *Lotus glaber* Mill. throughout the Flooding Pampa in Argentina may be attributed to ecotypic differentiation among populations. In order to confirm such genetic variation, two morphologically distinct populations from contrasting soil types, particularly in the phosphorus (P) status, were compared in their response to a different P supply. Plants were cloned by stem cuttings and transplanted to a common soil fertilized with increasing amounts of P. Morphological characters including number of shoots, leaf length and width, as well as the leaf length/width ratio, remained different in spite of P supply. Biomass P content, tissue P concentration and the percentage of root length infected by mycorrhizae associated with P uptake, were similar between populations. However, both populations showed a different growth response to P fertilization in spite of being cultivated on the same soil. Consequently, they differed in the efficiency of P utilization (E), which is probably under genetic control. These results produce evidence in support of the existence of ecotypes for *L. glaber* adapted to local soil conditions.

**KADE M., PAGANI E.A. and MENDOZA R.E.** 2003. A morphological study of populations of *Lotus glaber* Mill. (Fabaceae). *Agronomie*, **23**, 203-207.

The pasture legume *Lotus glaber* Mill. has colonized the Flooding Pampa (Argentina) in spite of high environmental heterogeneity. Morphological characters of plants from different populations were compared to evaluate if plastic or genetic differentiation could have contributed to such a widespread geographic distribution. Plants were collected at random from six different sites and grown in a greenhouse for five months. Clonal replicates of those plants were cultivated in pots on a native and a common soil. Populations were replicated three times and each replicate (pot) had three plants from the same clone. The number of primary shoots, internode length, leaf length and width and shoot length were recorded and the significance of population mean differences determined. All characters, except for leaf width, differed between populations on each sampling in both sets of experiments. This variability is therefore best explained by genetic differentiation of the populations.

**KRUSELL L., MADSEN L.H., GENUA A., SZCZYGLOWSKI K., AUBERT G., DUC G., SATO S., TABATA S., DE BRUIJN F., PAJUELO E., SANDAL N. and STOUGAARD J.** 2002. Shoot control of root development and nodulation is mediated by a receptor-like kinase. *Nature*, **420**, 422-426.

In legumes, root nodule organogenesis is activated in response to morphogenic lipochitin oligosaccharides that are synthesized by bacteria, commonly known as rhizobia. Successful symbiotic interaction results in the formation of highly specialized organs called root nodules, which provide a unique environment for symbiotic nitrogen fixation. In wild-type plants the number of nodules is

regulated by a signalling mechanism integrating environmental and developmental cues to arrest most rhizobial infections within the susceptible zone of the root. Furthermore, a feedback mechanism controls the temporal and spatial susceptibility to infection of the root system. This mechanism is referred to as autoregulation of nodulation, as earlier nodulation events inhibit nodulation of younger root tissues. *Lotus japonicus* plants homozygous for a mutation in the *hypernodulation aberrant root (har1)* locus escape this regulation and form an excessive number of nodules. Here we report the molecular cloning and expression analysis of the *HAR1* gene and the pea orthologue, *Pisum sativum*, *SYM29*. *HAR1* encodes a putative serine/threonine receptor kinase, which is required for shoot-controlled regulation of root growth, nodule number, and for nitrate sensitivity of symbiotic development.

**LEEP R.**, JERANYAMA P., MIN D.H. and DIETZ T. 2002. Grazing effects on herbage mass and composition in grass-birdsfoot trefoil mixtures. *Agronomy Journal*, **94**, 1257–1262.

Grass–legume mixtures have the ability to supply more consistent forage yields across a wide range of environments throughout the grazing season than do grass monocultures. The suitability of diverse grass species in binary mixtures with birdsfoot trefoil (*Lotus corniculatus* L.) in rotational stocking systems has not been extensively studied. The objective of this study was to evaluate binary mixtures of five cool-season grasses with the birdsfoot trefoil cultivar Norcen for herbage mass, botanical composition, and cattle (*Bos taurus*) grazing preference under a rotational stocking. Experiments were established at Lake City and Chatham, MI, in 1994. Binary mixtures were grazed for 2 yr with beef or dairy cows three times yearly at predetermined periods from spring to fall. Total herbage dry mass production ranged from 3 to 10 Mg ha<sup>-1</sup> yr<sup>-1</sup> over two years and locations. The grass fraction in binary mixtures was 327 to 946 g kg<sup>-1</sup> in swards over two years and locations. Perennial ryegrass (*Lolium perenne* L.) failed to persist at Lake City, probably due to less consistent snow cover. Birdsfoot trefoil fraction was highest in binary mixtures with smooth brome (*Bromus inermis* Leyss) and timothy (*Phleum pratense* L.). Binary mixtures with orchardgrass (*Dactylis glomerata* L.) and tall fescue (*Festuca arundinacea* Schreb.) produced the highest herbage biomass but were less preferred by grazing animals while binary mixtures with timothy and smooth brome were associated with the highest apparent herbage utilization at both locations (84–100%).

Full text at <http://agron.scijournals.org/cgi/content/full/94/6/1257>

**LEEP R.**, ANDRESEN J.R. and JERANYAMA P. 2001. Fall Dormancy and Snow Depth Effects on Winterkill of Alfalfa. *Agronomy Journal*, **93**, 1142–1148.

Received for publication December 11, 2000. The lack of a definitive method to assess winter hardiness in alfalfa (*Medicago sativa* L.) remains a challenge in the north-central region of the USA where winterkill of alfalfa can be severe. The reliability of fall dormancy ratings for describing alfalfa cultivar susceptibility to winter injury and the role of snow depth in moderating temperatures near the plant were investigated at Chatham, MI on a Chatham Stony loam (Typic Haplorthod). Four cultivars were selected with a range of fall dormancy ratings: ‘Nitro’, ‘Magnum IV’, ‘Saranac’, and ‘Vernal’. The cultivars were planted in 1993–1994, 1994–1995, and 1995–1996 seasons in plots over which 0-, 10-, and 20-cm winter snow depths were maintained. Temperatures were monitored for each plot, and stand counts were made each fall and spring to assess winter injury. Nitro suffered the most winterkill across snow cover treatments. The total yield range was 0 to 9 Mg ha<sup>-1</sup> in the absence of a snow cover and 0.4 to 12 Mg ha<sup>-1</sup> for a snow depth of at least 10 cm, except in 1996. Extreme minimum canopy-level (6 cm) temperatures for 10-cm snow depth averaged over three winter seasons were 12.1°C higher than the 0-cm snow cover treatment, which translated into higher yields. The results suggest that snow cover of 10 cm adequately protects alfalfa from winter injury. Cultivars within the same fall dormancy rating did not necessarily perform similarly, suggesting the need to develop other methods for assessing winter survival.

Full text at <http://agron.scijournals.org/cgi/content/full/93/5/1142>

MADSEN L.H., COLLINS N.C., RAKWALSKA M., BACKES G., SANDAL N., KRUSELL L., JENSEN J., WATERMAN E.H., JAHOOOR A., YALIFFE M., PRYOR A.J., LANGRIDGE P., SCHULTZE-LEFERT P. and **STOUGAARD J.** 2003. Barley disease resistance gene analogs of the NBS-LRR class – identification and mapping. *Molecular Genetics and Genomics*, **269**, 150-161.

The majority of verified plant disease resistance genes isolated to date are of the NBS-LRR class, encoding proteins with a predicted nucleotide binding site (NBS) and a leucine-rich repeat (LRR) region. We took advantage of the sequence conservation in the NBS motif to clone, by PCR, gene fragments from barley representing putative disease resistance genes of this class. Over 30 different resistance gene analogs (RGAs) were isolated from the barley cultivar Regatta. These were grouped into 13 classes based on DNA sequence similarity. Actively transcribed genes were identified from all classes but one, and cDNA clones were isolated to derive the complete NBS-LRR protein sequences. Some of the NBS-LRR genes exhibited variation with respect to whether and where particular introns were spliced, as well as frequent premature polyadenylation. DNA sequences related to the majority of the barley RGAs were identified in the recently expanded public rice genomic sequence database, indicating that the rice sequence can be used to extract a large proportion of the RGAs from barley and other cereals. Using a combination of RFLP and PCR marker techniques, representatives of all barley RGA gene classes were mapped in the barley genome, to all chromosomes except 4H. A number of the RGA loci map in the vicinity of known disease resistance loci, and the association between RGA S-120 and the nematode resistance locus Ha2 on chromosome 2H was further tested by co-segregation analysis. Most of the RGA sequences reported here have not been described previously, and represent a useful resource as candidates or molecular markers for disease resistance genes in barley and other cereals.

MADSEN E.B. MADSEN L.H., RADUTOIU S., OLBRYT M., RAKWALSKA M., SZCZYGLOWSKI K., KANEKO T., SATO S., TABATA S., SANDAL N. and **STOUGAARD J.** 2003. A receptor-like kinase gene involved in legume perception of rhizobial signal molecules. *Nature*, **425**, 637-640.

Plants belonging to the legume family develop nitrogen-fixing root nodules in symbiosis with bacteria commonly known as rhizobia. The legume host encodes all of the functions necessary to build the specialized symbiotic organ, the nodule, but the process is elicited by the bacteria. Molecular communication initiates the interaction, and signals, usually flavones, secreted by the legume root induce the bacteria to produce a lipochitin-oligosaccharide signal molecule (Nod-factor), which in turn triggers the plant organogenic process. An important determinant of bacterial host specificity is the structure of the Nod-factor, suggesting that a plant receptor is involved in signal perception and signal transduction initiating the plant developmental response. Here we describe the cloning of a putative Nod-factor receptor kinase gene (NFR5) from *Lotus japonicus*. NFR5 is essential for Nod-factor perception and encodes an unusual transmembrane serine/threonine receptor-like kinase required for the earliest detectable plant responses to bacteria and Nod-factor. The extracellular domain of the putative receptor has three modules with similarity to LysM domains known from peptidoglycan-binding proteins and chitinases. Together with an atypical kinase domain structure this characterizes an unusual receptor-like kinase.

MCKENZIE D.B., **PAPADOPOULOS Y.A.** and MCRAE K.B. 2004. Harvest management affects yield and persistence of birdsfoot trefoil (*Lotus corniculatus* L.) in cool summer climates. *Canadian Journal of Plant Science*, **84**, 525-528.

Two studies determined the effect of birdsfoot trefoil harvest managements on persistence, productivity, and species composition under cool summer growing conditions. Single and double cut managements were harvested at 10, 50, and 100% bloom; triple cuts and simulated grazing were also evaluated. Double cut harvest management produced considerably more dry matter yield (DMY) in the first production year, but its advantage over single cuts in the second year depended on bloom at harvest. Harvesting at 10% bloom for single (and perhaps double) cuts appears to be the best system based on DMY, trefoil content, and stand density.

**NAGAR P.S.** and PANDYA S.M. 2002. *Stylosanthes hamatus* (Linn.) Taub. (Papilionaceae): A new record to the flora of Gujarat. *Bomb. Nat. Hist. Soc.*, **99**, 363-364. (With one text figure).

**NAGAR P.S.** and PANDYA S.M. 2002. Addition to the flora of Saurashtra, Gujarat, India. *J.Econ. Taxon.Bot.*, **26**, 75-77.

**NAGAR P.S.** Floristic Diversity of Barda Hills and its surroundings. Scientific Publishers, Jodhpur. (Book, Publication in progress).

**NAGAR P.S.** Biodiversity of Barda Wildlife Sanctuary, GEER Foundation, Gandhinagar, Gujarat, India. (Book, Publication in progress).

**NAGAR P.S.** Medicinal plants of Gujarat, GEER Foundation, Gandhinagar, Gujarat, India. (Book, Publication in progress).

NIKOLIC R. and MITIC N. 2003. Morphological changes in atypical bird's foot trefoil plants obtained during genetic transformation by *Agrobacterium*. *Acta Biologica Jugoslavica, Series F, Genetics. (Acta Biolog. Jugosl. F G)*, **35**, 177-185.

PACIOS-BRAS C., SCHLAMANN H.R.M., BOOT K., ADMIRAAL P., LANGERAK J.M., **STOUGAARD J.** and SPAINK H.P. 2003. Auxin distribution in *Lotus japonicus* during root nodule development. *Plant Molecular Biology*, **52**, 1169-1180.

For this work, *Lotus japonicus* transgenic plants were constructed expressing a fusion reporter gene consisting of the genes beta-glucuronidase (*gus*) and green fluorescent protein (*gfp*) under control of the soybean auxin-responsive promoter GH3. These plants expressed GUS and GFP in the vascular bundle of shoots, roots and leaflets. Root sections showed that in mature parts of the roots GUS is mainly expressed in phloem and vascular parenchyma of the vascular cylinder. By detecting GUS activity, we describe the auxin distribution pattern in the root of the determinate nodulating legume *L. japonicus* during the development of nodulation and also after inoculation with purified Nod factors, N-naphthylphthalamic acid (NPA) and indoleacetic acid (IAA). Differently than white clover, which forms indeterminate nodules, *L. japonicus* presented a strong GUS activity at the dividing outer cortical cells during the first nodule cell divisions. This suggests different auxin distribution pattern between the determinate and indeterminate nodulating legumes that may be responsible of the differences in nodule development between these groups. By measuring of the GFP fluorescence expressed 21 days after treatment with Nod factors or bacteria we were able to quantify the differences in GH3 expression levels in single living roots. In order to correlate these data with auxin transport capacity we measured the auxin transport levels by a previously described radioactive method. At 48 h after inoculation with Nod factors, auxin transport showed to be increased in the middle root segment.

The results obtained indicate that *L. japonicus* transformed lines expressing the GFP and GUS reporters under the control of the GH3 promoter are suitable for the study of auxin distribution in this legume.

**PEDROSA A., SANDAL N., STOUGAARD J., SCHWEITZER D. and BACHMAIR A. 2002.**

Chromosomal map of the model legume *Lotus japonicus*. *Genetics*, **161**, 1661-1672.

*Lotus japonicus* is a model plant for the legume family. To facilitate map-based cloning approaches and genome analysis, we performed an extensive characterization of the chromosome complement of the species. A detailed karyotype of *L. japonicus* Gifu was built and plasmid and BAC clones, corresponding to genetically mapped markers (see the accompanying article by Sandal *et al.* 2002, this issue), were used for FISH to correlate genetic and chromosomal maps. Hybridization of DNA clones from 32 different genomic regions enabled the assignment of linkage groups to chromosomes, the comparison between genetic and physical distances throughout the genome, and the partial characterization of different repetitive sequences, including telomeric and centromeric repeats. Additional analysis of *L. filicaulis* and its F<sub>1</sub> hybrid with *L. japonicus* demonstrated the occurrence of inversions between these closely related species, suggesting that these chromosome rearrangements are early events in speciation of this group.

Free text at <http://www.genetics.org/cgi/content/full/161/4/1661>

**PERRY J.A., WANG T.L., WELHAM T.J., GARDNER S., PIKE J.M., YOSHIDA S., PARNISKE M.**

2003. A TILLING reverse genetics tool and a web-accessible collection of mutants of the legume *Lotus japonicus*. *Plant Physiology* **131**, 866-871.

Full text at <http://www.plantphysiol.org/cgi/content/full/131/3/866>

**QUAEDVLIEG N.E.M., SCHLAMMAN H.R.M., ADMIRAAL P.C., WIJTING S.E., STOUGAARD J.**

and SPAINK H.P. 1998. Fusions between green fluorescent protein and  $\beta$ -glucuronidase as sensitive and vital bifunctional reporters in plants. *Plant Molecular Biology*, **37**, 715-727.

By fusing the genes encoding green fluorescent protein (GFP) and beta-glucuronidase (GUS) we have created a set of bifunctional reporter constructs which are optimized for use in transient and stable expression studies in plants. This approach makes it possible to combine the advantage of GUS, its high sensitivity in histochemical staining, with the advantages of GFP as a vital marker. The fusion proteins were functional in transient expression studies in tobacco using either DNA bombardment or potato virus X as a vector, and in stably transformed *Arabidopsis thaliana* and *Lotus japonicus* plants. The results show that high level of expression does not interfere with efficient stable transformation in *A. thaliana* and *L. japonicus*. Using confocal laser scanning microscopy we show that the fusion constructs are very suitable for promoter expression studies in all organs of living plants, including root nodules. The use of these reporter constructs in the model legume *L. japonicus* offers exciting new possibilities for the study of the root nodulation process.

**RADUTOIU S., MADSEN L.H, MADSEN E.B, FELLE H., UMEHARA Y., GRØNLUND M.,**

KANEKO T., SATO S., TABATA S., SANDAL N. and **STOUGAARD J.** 2003. Plant recognition of symbiotic bacteria requires two LysM receptor-like kinases. *Nature*, **425**, 585-592.

Although most higher plants establish a symbiosis with arbuscular mycorrhizal fungi, symbiotic nitrogen fixation with rhizobia is a salient feature of legumes. Despite this host range difference, mycorrhizal and rhizobial invasion shares a common plant-specified genetic programme controlling the early host interaction. One feature distinguishing legumes is their ability to perceive rhizobial-specific signal molecules. We describe here two LysM-type serine/threonine receptor kinase genes, NFR1 and NFR5, enabling the model legume *Lotus japonicus* to recognize its bacterial microsymbiont

*Mesorhizobium loti*. The extracellular domains of the two transmembrane kinases resemble LysM domains of peptidoglycan- and chitin-binding proteins, suggesting that they may be involved directly in perception of the rhizobial lipochitin-oligosaccharide signal. We show that NFR1 and NFR5 are required for the earliest physiological and cellular responses to this lipochitin-oligosaccharide signal, and demonstrate their role in the mechanism establishing susceptibility of the legume root for bacterial infection.

SANDAL N., KRUSELL L., RADUTOIU S., OLBRYT M., **PEDROSA A.**, STRACKE S., SATO S., KATO T., TABATA S., PARNISKE P., BACHMAIR A., KETELSEN T. and STOUGAARD J. 2002. A Genetic Linkage Map of the Model Legume *Lotus japonicus* and Strategies for Fast Mapping of New Loci. *Genetics*, **161**, 1673-1683.

A genetic map for the model legume *Lotus japonicus* has been developed. The F2 mapping population was established from an interspecific cross between *L. japonicus* and *L. filicaulis*. A high level of DNA polymorphism between these parents was the source of markers for linkage analysis and the map is based on a framework of amplified fragment length polymorphism (AFLP) markers. Additional markers were generated by restriction fragment length polymorphism (RFLP) and sequence-specific PCR. A total of 524 AFLP markers, 3 RAPD markers, 39 gene-specific markers, 33 microsatellite markers, and six recessive symbiotic mutant loci were mapped. This genetic map consists of six linkage groups corresponding to the six chromosomes in *L. japonicus*. Fluorescent *in situ* hybridization (FISH) with selected markers aligned the linkage groups to chromosomes as described in the accompanying article by Pedrosa *et al.* (2002, this issue). The length of the linkage map is 367 cM and the average marker distance is 0.6 cM. Distorted segregation of markers was found in certain sections of the map and linkage group I could be assembled only by combining colormapping and cytogenetics (FISH). A fast method to position genetic loci employing three AFLP primer combinations yielding 89 markers was developed and evaluated by mapping three symbiotic loci, *Ljsym1*, *Ljsym5*, and *Ljhar1-3*. Free text at <http://www.genetics.org/cgi/content/full/161/4/1673>

SCHAUSER L., HANDBERG K., SANDAL N., STILLER J., THYKJÆR T., PAJUELO E., NIELSEN A. and **STOUGAARD J.** 1998. Symbiotic mutants deficient in nodule establishment identified after T-DNA transformation of *Lotus japonicus*. *Molecular Genetics and Genomics*, **259**, 414-423.

Nitrogen-fixing root nodules develop on legumes as a result of an interaction between host plants and soil bacteria collectively referred to as rhizobia. The organogenic process resulting in nodule development is triggered by the bacterial microsymbiont, but genetically controlled by the host plant genome. Using T-DNA insertion as a tool to identify novel plant genes that regulate nodule ontogeny, we have identified two putatively tagged symbiotic loci, *Ljsym8* and *Ljsym13*, in the diploid legume *Lotus japonicus*. The *sym8* mutants are arrested during infection by the bacteria early in the developmental process. The *sym13* mutants are arrested in the final stages of infection, and ineffective nodules are formed. These two plant mutant lines were identified in progeny from 1112 primary transformants obtained after *Agrobacterium tumefaciens* T-DNA-mediated transformation of *L. japonicus* and subsequent screening for defects in the symbiosis with *Mesorhizobium loti*. Additional nontagged mutants arrested at different developmental stages were also identified and genetic complementation tests assigned all the mutations to 16 monogenic symbiotic loci segregating recessive mutant alleles. In the screen reported here independent symbiotic loci thus appeared with a frequency of approximately 1.5%, suggesting that a relatively large set of genes is required for the symbiotic interaction.

SCHAUSER L., ROUSSIS A., STILLER J. and **STOUGAARD J.** 1999. A plant regulator controlling development of symbiotic root nodules. *Nature*, **402**, 191-195.

Symbiotic nitrogen-fixing root nodules on legumes are founded by root cortical cells that de-differentiate and restart cell division to establish nodule primordia. Bacterial microsymbionts invade these primordia through infection threads laid down by the plant and, after endocytosis, membrane-enclosed bacteroids occupy cells in the nitrogen-fixing tissue of functional nodules. The bacteria excrete lipochitin oligosaccharides, triggering a developmental process that is controlled by the plant and can be suppressed. Nodule inception initially relies on cell competence in a narrow infection zone located just behind the growing root tip. Older nodules then regulate the number of nodules on a root system by suppressing the development of nodule primordia. To identify the regulatory components that act early in nodule induction, we characterized a transposon-tagged *Lotus japonicus* mutant, *nin* (for nodule inception), arrested at the stage of bacterial recognition. We show that *nin* is required for the formation of infection threads and the initiation of primordia. NIN protein has regional similarity to transcription factors, and the predicted DNA-binding/dimerization domain identifies and typifies a consensus motif conserved in plant proteins with a function in nitrogen-controlled development.

**STOUGAARD J.** 2000. Regulators and regulation of root nodule development. *Plant Physiology*, **124**, 531-540. [Review]

Full text at <http://www.plantphysiol.org/cgi/content/full/124/2/531>

**STOUGAARD J.** 2001. *Lotus japonicus*. *In* Encyclopaediae of Genetics, Academic press, pp 1121-1122. [Review]

**STOUGAARD J.** 2001. Genetics and genomics of root symbiosis. *Current Opinion in Plant Biology*, **4**, 328-336. [Review]

Model genetics and genomics have been developed as tools for studying the third largest family of flowering plants, the Leguminosae, which includes important crop plants. Functional genomics strategies for the global analysis of gene expression, the elucidation of pathways and reverse genetics are established. These approaches provide new possibilities for investigating rhizobial as well as mycorrhizal endosymbiosis. Plant genes with central functions in these mutualistic interactions have been identified by positional cloning and gene tagging. With progress in *Lotus japonicus* genome sequencing, which was recently initiated by Japanese researchers, comparative genomics will contribute to our understanding of symbiosis, pathogenesis and the evolution of plant genome.

**STOUGAARD J., SZCZYGLOWSKI K., DE BRUIJN F.J. and PARNISKE M.** 1999. Genetic nomenclature guidelines for the model legume *Lotus japonicus*. *Trends in Plant Science*, **4**, 300-301. [Manual]

**STRACKE S., KISTNER C., YOSHIDA S., MULDER L., SATO S., KANEKO T., TABATA S., SANDAL N., STOUGAARD J., SZCZYGLOWSKI K. and PARNISKE M.** 2002. A plant receptor-like kinase required for both bacterial and fungal symbiosis. *Nature*, **417**, 959-962.

Most higher plant species can enter a root symbiosis with arbuscular mycorrhizal fungi, in which plant carbon is traded for fungal phosphate. This is an ancient symbiosis, which has been detected in fossils of early land plants. In contrast, the nitrogen-fixing root nodule symbioses of plants with bacteria evolved more recently, and are phylogenetically restricted to the rosoid I clade of plants. Both symbioses rely on partially overlapping genetic programmes. We have identified the molecular basis for this convergence by cloning orthologous SYMRK ('symbiosis receptor-like kinase') genes from *Lotus* and pea, which are required for both fungal and bacterial recognition. SYMRK is predicted to

have a signal peptide, an extracellular domain comprising leucine-rich repeats, a transmembrane and an intracellular protein kinase domain. Lotus SYMRK is required for a symbiotic signal transduction pathway leading from the perception of microbial signal molecules to rapid symbiosis-related gene activation. The perception of symbiotic fungi and bacteria is mediated by at least one common signalling component, which could have been recruited during the evolution of root nodule symbioses from the already existing arbuscular mycorrhiza symbiosis.

**WANG T.L.**, DOMONEY C., HEDLEY C.L., CASEY R. and GRUSAK M.A. 2003. Can we improve the nutritional quality of legume seeds? *Plant Physiology*, **131**, 886-891.

Full text at <http://www.plantphysiol.org/cgi/content/full/131/3/886>

WEGEL E., SCHAUSER L., SANDAL N., **STOUGAARD J.** and PARNISKE M. 1998. Mycorrhiza mutants of *Lotus japonicus* define genetically independent steps during symbiotic infection. *Molecular Plant Microbe Interactions*, **11**, 933-936.

WOPEREIS J., PAJUELO E., DAZZO F.B., JIANG Q., GRESSHOFF P.M., DE BRUIJN F.J., **STOUGAARD J.** and SZCZYGLOWSKI K. 2000. Short root mutant of *Lotus japonicus* with a dramatically altered symbiotic phenotype. *Plant Journal*, **23**, 97-114.

Legume plants carefully control the extent of nodulation in response to rhizobial infection. To examine the mechanism underlying this process we conducted a detailed analysis of the *Lotus japonicus* hypernodulating mutants, har1-1, 2 and 3 that define a new locus, HYPERNODULATION ABERRANT ROOT FORMATION (Har1), involved in root and symbiotic development. Mutations in the Har1 locus alter root architecture by inhibiting root elongation, diminishing root diameter and stimulating lateral root initiation. At the cellular level these developmental alterations are associated with changes in the position and duration of root cell growth and result in a premature differentiation of har1-1 mutant root. No significant differences between har1-1 mutant and wild-type plants were detected with respect to root growth responses to 1-aminocyclopropane-1-carboxylic acid, the immediate precursor of ethylene, and auxin; however, cytokinin in the presence of AVG (aminoethoxyvinylglycine) was found to stimulate root elongation of the har1-1 mutant but not the wild-type. After inoculation with *Mesorhizobium loti*, the har1 mutant lines display an unusual hypernodulation (HNR) response, characterized by unrestricted nodulation (hypernodulation), and a concomitant drastic inhibition of root and shoot growth. These observations implicate a role for the Har1 locus in both symbiotic and non-symbiotic development of *L. japonicus*, and suggest that regulatory processes controlling nodule organogenesis and nodule number are integrated in an overall mechanism governing root growth and development.

Full text at <http://www.blackwell-synergy.com/links/doi/10.1046/j.1365-313x.2000.00799.x/full/>

ZAGROBELNY M., **BAK S.**, RASMUSSEN A.V., JØRGENSEN B., NAUMANN C.M., LINDBERG MØLLER B. 2004. Cyanogenic glucosides and plant-insect interactions. *Phytochemistry*, **65**, 293-306. [Review]

Cyanogenic glucosides are phytoanticipins known to be present in more than 2500 plant species. They are considered to have an important role in plant defense against herbivores due to bitter taste and release of toxic hydrogen cyanide upon tissue disruption. Some specialized herbivores, especially insects, preferentially feed on cyanogenic plants. Such herbivores have acquired the ability to metabolize cyanogenic glucosides or to sequester them for use in their predator defense. A few species of Arthropoda (within Diplopoda, Chilopoda, Insecta) are able to de novo synthesize cyanogenic glucosides and, in addition, some of these species are able to sequester cyanogenic glucosides from

their host plant (Zygaenidae). Evolutionary aspects of these unique plant–insect interactions with focus on the enzyme systems involved in synthesis and degradation of cyanogenic glucosides are discussed.

ZHANG S., SANDAL N., POLOWICK P.L., STILLER J., **STOUGAARD J.** and FOBERT P.R. 2003. Proliferating Floral Organs (*PFO*) a *Lotus japonicus* gene required for specifying floral meristem determinacy and organ identity, encodes an F-box protein. *Plant Journal*, **33**, 607-619.

To study flower development in the model legume *Lotus japonicus*, a population of transgenic plants containing a maize transposable element (*Ac*) in their genome was screened for floral mutants. One mutation named *proliferating floral organs* (*pfo*) causes plants to produce a large number of sepal-like organs instead of normal flowers. It segregates as a single recessive Mendelian locus, and causes sterility. Scanning electron microscopy revealed that *pfo* affects the identity, number and arrangement of floral organs. Sepal-like organs form in the first whorl, and secondary floral meristems are produced in the next whorl. These in turn produce sepal-like organs in the first whorl and floral meristems in the second whorl, and the process is reiterated. Petals and stamens are absent while carpels are either absent or reduced. The *pfo* phenotype was correlated with the presence of an *Ac* insertion yielding a 1.6-kb *HindIII* restriction fragment on Southern blots. Both the mutant phenotype and this *Ac* element are unstable. Using the transposon as a tag, the *Pfo* gene was isolated. Conceptual translation of *Pfo* predicts a protein containing an F-box, with high overall similarity to the *Antirrhinum* FIMBRIATA, *Arabidopsis* UNUSUAL FLORAL ORGANS and *Pisum sativum* Stamina pistilloida proteins. This suggests that Pfo may regulate floral organ identity and meristem determinacy by targeting proteins for ubiquitination.